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THE STRUCTURE OF THE CHROMOSOMES IN *TRADESCANTIA VIRGINICA* L.

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As a result of studies on the nuclei of *Tradescantia virginica* L., I am taking up previously discussed questions relating to the structure of the chromosome.

That there is still little agreement in regard to this important problem is shown by the relatively recent papers of Bonnevie (1908), von Herwerden (1910), Nawaschin (1911), Erhard (1910), Vejdovsky (1912), Lundegårdh (1912*a*), Suessenguth (1921), and Martens (1922).

An examination of the literature discloses a great volume of data bearing on this important subject, which perhaps originated with the much quoted paper of Balbiani (1881), in which he first called attention to striations on the chromatic filaments of the nucleus.

Throughout this mass of literature, figures are given which represent appearances obtained by different methods of fixing, staining, and imbedding. As noted, the interpretations of these microscopic appearances in the chromosome vary widely, and are frequently influenced too much by attempts to harmonize the descriptions of them with what is believed to be true regarding their behavior.

I shall restrict my description to certain stages: in the pollen mother cells, those from diakinesis (Häcker, 1905), to the arrangement of the chromosomes on the equatorial plate (*Sternfigur* of Flemming) just prior to separation; and further, both in the pollen mother cells and the somatic cells, to the stages after complete separation has been effected, *i.e.*, from the beginning of the anaphase to that telophase stage just prior to the reformation of the daughter nuclear membranes. I shall omit the series of processes involving the separation of the chromosomes during the metaphases.

The more intimate structure of the chromatic *Fäden* or *Schleifen* has been much discussed for every point between these stages, but, in following the older authors, it is frequently difficult to know to which particular stage of the division figure the writer refers. Naturally, such ambiguities

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in the older literature are to be expected, for at the time, the steps in mitosis were not so specifically defined. In this description, reference is made to the individual chromosome and not to tetrads as such.

My material was prepared by the modernized aceto-carmine method as described by Belling (1921*b*). Material was also fixed in Flemming's medium fluid followed by sectioning and staining. In addition to these methods, pollen mother cells were teased out on a slide into a 3 percent cane-sugar solution and studied in the living condition. The staminate hairs were suspended in 3 percent cane sugar, and the course of the divisions could be followed as described by Strasburger (Practicum, p. 604) for the staminate hairs of Tradescantia and by Lundegårdh (1912*b*) for root-tip divisions. The temperature was satisfactory when between 75° and 80° F.

The drawings of Plate XXIX were made entirely from aceto-carmine preparations. This is certainly a most valuable reagent for studying the chromatic elements of a cell to the exclusion of others. The solution may be employed in any dilution, and I found that for general purposes one drop of the modernized Schweigger-Seidel (1868) preparation added to one drop of water on a slide gave almost instantaneous staining.

The fluid acts as a swelling agent, so that the preparations in a one-to-one dilution go to pieces in about a week. For this reason, it is desirable to know how soon after treatment the chromosomes will show any particular stage in the swelling.

The mother cells were teased from the anthers into a drop of water on a slide to which then was added, ordinarily, a drop of the aceto-carmine. The mount was made as described by Belling (1921*b*), who first instructed me in the method. It was noted, in cases in which the stain was run under the cover glass, that about ten seconds were required to bring the chromosomes into the sharpest definition.

The structure came out with the first swelling, somewhat as does the image on a photographic plate, without any perceptible change either in the form or in the position of the elements from that seen in the living condition. The first perceptible change can be noticed in about an hour, when the elements may or may not appear a trifle swollen. When slight swelling occurs, it affords considerable advantage for studying the morphological composition of the chromosomes.

The figures given on the accompanying plates were, for the most part, made shortly after fixation, *i.e.*, after some slight swelling had occurred. The effect with the reagent noted, while apparently altering the indices of refraction of the cell elements and thus increasing the sharpness of their differentiation, leaves the chromosomes, especially, quite unaltered except for the probable slight hydration.

Most of the currently used killing agents, and especially the practice of hardening and imbedding, tend to greater density and even shrinkage of the cell structures. The use of such a fixative as this is especially advantageous as a check and for comparison.

The structures here are more or less obscured and are, in fact, rarely seen in the material prepared by the standard killing solutions and imbedding methods. A comparison of Tradescantia prepared by these two methods convinces me that, in the sectioned material, the finer details of the living cells are lost by slight fusions, shrinkage, and distortions, which leave perhaps the relative proportions as a whole unimpaired.

By dilution technique, any desired degree of staining intensity may be obtained ranging from none or the natural hyaline to that of deep color. As stated, the most frequently used dilution was, perhaps, one drop of aceto-carmine to one drop of water. The rate of disintegration suffered by the mounts prepared with this dilution was usually so rapid that one could not study the preparation for several consecutive days. Weaker mixtures were therefore devised.

Dilutions were made in a series of test tubes, so that tube *A* contained a one-to-one mixture; tube *B*, a one-in-four; tube *C*, a one-in-eight; tube *D*, a one-in-sixteen; and tube *E*, a one-in-thirty-two. If the tubes are cleaned of all foreign inorganic and organic matter, the stain will not precipitate and the tubes may be kept corked. Paraffined corks should be prepared, otherwise evaporation may be considerable. For most anthers, tube *E* contained too much water in proportion to acetic acid so that the effects of toxic stimulus generally appeared.

This method was found preferable to one in which pipettes graduated to hundredths of a cubic centimeter were employed for making the dilutions directly on the slide. Too much inaccuracy resulted from the latter practice.

The 1: 16 dilution was very satisfactory, and permitted one to observe the cells unstained for a long period of time, then through all intermediate stages of slow color absorption till at last the chromatin contents are of a deep rose color. This latter may require anywhere from six to twenty hours, since the contents of the anther sacs do not all react alike to the staining fluid. Some take up the stain more slowly than others. I found that with weaker dilutions the cells would often burst; in order to overcome this the dilutions were made more nearly isotonic by the addition of cane-sugar solution instead of water.

The dividing pollen mother cells of *Tradescantia virginica* L. treated as above described show clearly that the chromatic elements are composed of bodies roughly similar to those described by Balbiani (1881) and by Pfitzner (1882). The shape and size of these granules and their arrangement or distribution in the chromosome have not, however, been adequately described.

In figures 11 and 13, Plate XXIX, some of the strands are seen to be apparently made up of two rows of granules side by side. By numerous authors, these granules have been spoken of as *chromomeres*, a term introduced by Fol (1891). Eisen (1900) distinguishes *chromioles* as components of chromomeres. I also consider the bodies making up the chromosome

to be compound, but do not find a definite number of the bodies that he defines as chromioles. O. Hertwig (1906) applied the term *chromiole* to the *Scheibchen* of Chironomus nuclear filaments.

The shape of the chromomeres varies from spherical to oval, to cubical, with or without rounded edges and corners, and finally to irregular. As generally found, they are *doubtless aggregates* and are made up of smaller elements which again may be found subdivided into varying grades of smaller particles. It has been generally held (Mottier, 1907, p. 336; Miyake, 1905; and others) that these chromomeres are not the ultimate units of the chromatin itself. Strasburger says:

> Eine Ide ist wie wir sahen, nicht der letzte der im Kern festzustellenden Struckturelemente; sie geht aus der Vereinigung kleiner Gebilde hervor (1905, p. 53).

Conversely, then, as I consider it, the ultimate chromatic particles agglutinate into groups, these unite to form an aggregate of groups, and the group aggregates unite to form a chromomere. The whole process may be a series of successive agglutinations which involve, in the first instance, the migration of the minute particles through the homogeneous matrix (*Zwischensubstanz*) of the chromosome, *i.e.*, the linin.

The term *Id* has been avoided because it is not desired to associate these bodies with Weismann's (1885) conceptions, nor indeed is it intended to identify the chromomeres as described with any of the theoretical loci recently developed in the genetical field (see Mottier, 1907, p. 336).

I consider the unit of these agglomerates to be the ultimate microscopic chromatin particle as observed usually in *fixed material*, and as discussed by Wilson (1900, p. 37). Meyer (1920) has recently formulated the most specific hypothesis yet advanced as to the ultimate ultra-microscopic composition of the chromatin and cell contents. He assumes as essential constituents of the cell at least three types of so-called *Vitüle*, cytoplasmic vitules, nuclear vitules, and trophoplasmic vitules. These vitules are also extremely complex and are assumed to be made up of so-called *mions*. The mass of an electron is said to be 2,000 times smaller than that of a hydrogen atom. Meyer assumes the mion to be 2,000 times smaller than the electron. I shall take up this point in another paper.

In the mother cells, the linin appears to be distinct in composition from the linin of the achromatic spindle, since, in preparations stained with aceto-carmine, it appears rather dense and of a most delicate straw to rose color, whereas no element of the achromatic spindle is either stained or perceptible. On the other hand, in preparations of the stamen hair, the elements of the achromatic spindle are clearly visible both in stained and in unstained material but only between anaphase and telophase. The surfaces of the *Zwischensubstanz* are clearly marked, not only by a very delicate color differentiation, but also by their relative refractive indices.

How many grades in size there may be in the subdivision of the chromomere is not clear. Figure 8, Plate XXIX, figures the construction as seen

in the aceto-carmine preparations. Here only two size differences could be clearly seen, and this represents the most that could be counted.

Strasburger (1904) assumes a compound construction for his *gamosomes* as he does for the *Ide*, but not quite in the same sense as presented in this paper. He says:

> Das Chromatin zieht sich aus den Lininfäden zurück und lässt sie als wenig tingierbare, zarte, perlschnurartig gegliederte Fäden zurück. Es bildet Körnchen, die sich um einzelne Zentren sammeln.

The chromomeres of figure 8, Plate XXIX, seem to be made up of four smaller bodies each, but this is of course an optical section. Careful focusing up and down shows others to lie beneath. I have seen these smaller bodies again subdivided, but this is not always a mathematically regular phenomenon, such as Miss Merriman (1904) has reported for Allium. I am convinced of the accuracy of her figures 35, 39, and 41, Plate XII, but do not feel that she is warranted, without further evidence, in drawing the conclusion that each subdivision of the chromosome proceeds by fours any more than Bonnevie (1908) would be justified in such a statement by reason of her figures 11, 16, and 22, Plate XIV.

The number of these subdivisions is variable. A careful study shows that the chromatic thread, contrary to the impression first given as it is seen in figure 13, Plate XXIX, is not made up of chromomeres side by side. The arrangement is more complex than this. In any given field of vision and with any group of chromosomes, the breadth of the individual chromosome, as well as that of any length of thread exclusive of constriction points, is constant, which would not be the case if they were made up of rows of bodies side by side, that is, if their real shape was ribbon-like.

Heidenhain (1907) figures chromosomes which appear ribbon-like in cross section (text fig. 1). I have not seen such forms either in the living material or in that fixed by any of the methods mentioned above.

In figures 3 and 4, Plate XXIX, an enlarged drawing of the ends of two threads is seen. Here one sees that the chromomeres are apparently arranged irregularly just inside the periphery of the linin cylinder. The surface of the cylinder may be nodular, irregular, or smooth, according to whether or not the chromomeres extend beyond the periphery.

The chromomeres shown in figure 10 project more than those in figure 5, so that the degree of irregularity presented by the chromosomal outline is a function of the degree of projection of these bodies. In the unstained and living chromosome, the surface has a punctate appearance, not only in the telophases but in the early anaphases or even before, so that, while Lundegårdh (1912*b*) has described a nodular chromosome structure in the telophases of the vegetative divisions, I would extend this description to include the reduction divisions and, in all cases, the chromosomes of the metaphases and anaphases. Some evidence of this is seen in figure 15, Plate XXX. Note the last chromosome at the bottom of the figure.

This photograph was taken from a stamen hair suspended in 3 percent cane-sugar solution and unstained. The chromosomes are living, and numerous ones appear quite smooth. It was found that the greater the density of the sugar solution, the greater was the apparent smoothness of the chromosome outlines.

The central axis of the chromosome remains clear of these bodies. One may say that its structure, in this sense, is tubular. To use a homely comparison, if one could fill a sausage skin with irregular pebbles in such a manner that the central axis would remain clear, and if the pebbles pressed against the skin so as to give it an irregular nodular outline, this would represent crudely the structure of the chromosome. The bounding membrane would, of course, represent merely the linin surface, and a truer description would perhaps regard the chromomeres as more or less imbedded in a linin cylinder as a continuous phase. Figure 19, Plate XXX, also from a living unstained preparation, shows, in the upper half, the ends of two chromosomes. In these two, the hollow structure is quite obvious even in the photograph. It was, naturally, much more so to the eye of the observer.

An outer chromosomal membrane such as Wenrich (1916) describes for Phrynottetix has never been observed by me in Tradescantia. Such a membrane could be demonstrated neither in the living chromosome in a sugar solution nor in any of the fixed material. For the present, I prefer to consider such figures as Lee's figure 4, Plate I, (1920) to be due to the action of fixing fluid which caused the chromatin to shrink, thereby leaving the achromatic substratum through which it was originally distributed unchanged in form. His vacuolated condition of the plant chromosome as distinguished from the solid structure of the animal chromosome must also be due to the fickleness of our preparation methods.

In figure 9, Plate XXIX, the chromosomes are about to be oriented on the equatorial plate. One observes here several whose longitudinal axes lie parallel with the optical axis of the microscope so that the ends are presented to the eye of the observer (*b*, fig. 9).

The relationship of the chromomeres, one to the other, is somewhat variable. Sometimes they show traces of a spiral arrangement (figs. 17, 18, Pl. XXX). At other times they are mostly paired, as in figure 16, or else irregularly spaced as in figures 11 and 13, Plate XXIX. In figure 12, chromomeres 2 and 3, 5 and 7, 10 and 12, 13 and 14, are located in the median optical plane of the chromosome and possibly opposite each other, so that the free spaces taken along the chromosomal axis represent the distances between them. The chromomeres 4, 6, 8, 11, and 15 of this figure overlie the hollow center and more or less overlie the other chromomeres.

This figure represents a chromosome that became freed from its mother cell. Part of the cell wall had been torn by the needle while being teased

from the anther sac, but when the cell was found the metaphase-plate figure was still in position and intact. By pressing successively on the cover glass with a fine, springy needle, the chromosomes were finally separated from the cytoplasm. By proper manipulations, often by merely tapping the microscope stage with a pencil, the specimens would roll over, thus permitting the observer to view all sides of the chromosomes. The chromosome in question was studied in this way, and it was then discovered that the chromomeres numbered 4, 6, 8, 11, and 15 were opposite others separated from them by the hollow center of the chromosome. The whole chromosome was found thus to have a rather regular four-rowed construction. (See Nawaschin, 1911.) That this is perhaps not a general condition is shown by the arrangement in figure 1, Plate XXIX, where chromomeres 4, 7, 8, and 20 seemed to be arranged in various positions with respect to the others. Here, again, the arrangement of those in the median optical plane of the chromosome appeared opposite.

In figure 2, the chromomeres have been numbered with reference to their distance from the eye-piece as determined by focusing up and down through the mass of the chromosome. Chromomere no. 1 is nearest to the eye; no. 14, the farthest from the eye. Those between are in successively intermediate positions.

It is considered that in figure 12, chromomere 16 is at the distal[1] end of the chromosome, *i.e.*, at the end away from the spindle attachment. Chromomeres 2, 5, 8, and 10 are therefore higher than 3, 7, 9, and 12. In the same figure, chromomeres 4, 6, 8, and 11 seem to have the planes of their sides somewhat oblique to the long axis of the chromosome. The arrangement as a whole simulates a segmented spiral, which is also the case in figure 5, notably, here, between chromomeres 10, 11, 12, and 13. On the other hand, one arguing for the view that the chromomeres are parts of fragmented disks and hence opposite will find, as noted, some evidence to support such a claim.

An understanding of the true construction of the chromosome is obviously very dependent upon a study of the segments in all possible optical planes. Let text figure 2 represent the cross section of a chromosome when the construction is assumed to consist of four rows of chromomeres arranged opposite to one another, and the line *po* the median optical plane. If the focus is adjusted to the line *xy*, the image of the chromosome will show numerous, practically solid, cross striations or bands. It was by carefully focusing up and down that chromomere *c*, figure 9, Plate XXIX, and the overlying chromomeres in figure 1 of the same plate were located. In the latter case, the specimen was rotated on the microscope stage by tapping, and its cross section while on end was studied. The band-like structure is seen here and there in figure 10, Plate XXIX, while in figure 9 the two-ranked chromomeres, as well as a band in a few cases, are seen, accord-

[1] Wenrich (1916).

ing to the focal depth at which the bodies are observed. (Compare the photographs on Plate XXX.)

This composite construction of the chromosome holds true for the chromosomes of the homoeotypic divisions. These chromosomes appear more gracefully modeled than do those of the heterotypic spindle. Figure 5 is a free-hand drawing of one of the second-division chromosomes, and is the same one as *a*, figure 6, Plate XXIX. The relations of the chromomeres seem to be the same here as already discussed, but they can be made out only under the most favorable conditions.

Figure 7 represents two free-hand drawings of the same chromosome. The upper drawing is of the top focus; the lower is at the bottom focus. From these drawings one obtains an idea of the irregularly shaped chromomeres that are sometimes encountered. One is able to trace the shapes of the bodies through the linin mass from top to bottom. The clear space or constriction in the center resulted from applying pressure to the cover glass while teasing the chromosomes into the suspension fluid, so that too much importance may not be attached to forms such as these.

The linin substratum seems to be of a jelly-like consistency, and if the chromosomes are crushed the chromomeres often fuse in a more or less solid mass. Varying degrees of contact from complete independence to complete fusion may be observed in cells injured by dissection instruments.

A Correlation of these Findings with Others of a Similar Nature

Balbiani (1881) first called attention to disks of chromatin in the nuclear filaments of salivary-gland cells in Chironomus larvae. These had been fixed in a mixture of ½ percent chromic- and acetic-acid solutions. The glands were treated with the reagent for a few minutes, washed in distilled water, and, after staining with methyl green, were mounted in glycerin.

A considerable amount of work on the same material has been done, principally by Lydig (1883), Carnoy (1884), Korschelt (1884), Erhard (1910), von Herwerden (1910), Bolsius (1911), and Alverdes (1912). All the authors agree on the compound nature of the threads but differ as to the details.

Balbiani considered these chromosome constituents to be disks arranged in a series with clear spaces filled with *Zwischensubstanz* separating them. He likens them to a series of red blood corpuscles placed end to end.

Lydig (1883) held that the dark transverse segments of the chromosome are themselves made up of smaller bodies, and that the lines separating these may make up something like longitudinal lines of division in the chromatic material. It may be noted that these more irregular lines of division agree in part with my findings as shown by several figures of Plates XXIX and XXX.

Korschelt held that the cross and other markings are due merely to the wrinkling of the chromosome surface.

Henle (1882) held that the striations seen by Balbiani and others were artifacts and the result of post-mortem coagulation, but Carnoy (1884) took exception to his contention:

On peut affirmer que le boyau de nucléine existe pendant la vie, tel que nous venons de le décrire. C'est en effet sur les cellules vivantes que nous l'avons étudié, fig. 66 et 67. Les boyaux si volumineux des insectes sont faciles à examiner de cette façon. On les voit parfaitement, même avec l'objectif DD, à l'intérieur du noyau; on peut y suivre les circonvolutions, et y distinguer nettement les stries dont nous parlerons tout-à-l'heure.

Objections may arise upon comparing these gland-cell nuclei with other nuclei involved in ordinary mitotic divisions, since, in connection with secretory activity, it is common to observe nuclei of irregular constitution and shapes such as those in the spinning cells of Arachnids—the spireme nuclei of Wilson (1900, p. 35)—or those found in the polymorphonuclear leucocytes. The structures under discussion are not confined to these cells. Carnoy (1884) described them for the plant *Paris quadrifolia* simultaneously with other forms (text fig. 3).

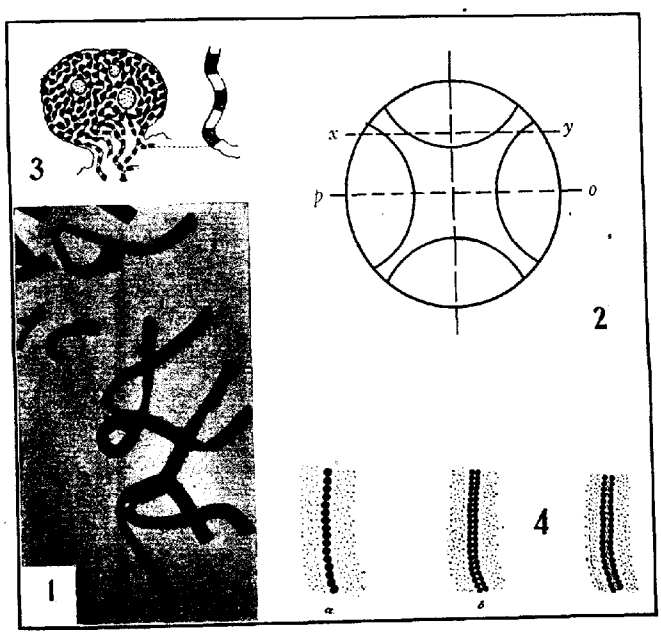

FIG. 1. Ribbon-like chromosomes figured by Heidenhain (1907). FIG. 2. Schematic representation of a quadripartite chromosomal cross section. FIG. 3. Chromomere construction as figured by Carnoy (1884) in *Paris quadrifolia*. FIG. 4. Pfitzner's conception of the splitting chromatic granules, taken from Flemming (1882).

Baranetzky (1880) first studied chromosome structure in *Tradescantia virginica* L. He notes that:

> Die Schärfe und die Regelmässigkeit des Baues ist oft so gross, dass beim ersten Anblick die Kernfäden unwillkürlich an die aus platten Zellen bestehenden Oscillarienfäden erinnern.

He further notes that the contours of the chromosome outlines are finely wavy owing to a slight constriction of the *Zwischensubstanz* between the disks. He describes the splitting of the chromosomal disks as taking place at right angles to the long axis of the chromosomes, and further states that the cleft begins at the surface of the disk and proceeds toward the center without good evidence that he has actually observed the process. Baranetzky also reports the same structure of the chromosomes for Agapanthus, Hemerocallis, Yucca, Hesperis, Lathyrus, and Pisum.

Erhard's (1910) aberrant ideas as to the relation of the chromatin and the nucleus need hardly be considered.

Pfitzner (1882) described the chromatic threads of the nuclei of many different tissues as being made up of granules, the "Pfitzner granules" (text fig. 4).

It is to be emphasized that there is practically no disagreement on the point that this construction obtains for the prophase stages. One of the first to point this out was Hermann in 1891. Almost every investigator has figured the chromatin bodies at this stage, notably Flemming (1879), Strasburger (1882), Bonnevie (1908), Vejdovsky (1912), and others too numerous to mention.

Flemming (1882), working with fixed material, recognized the granular composition of the chromosomes throughout the process of division (text fig. 5). His figures of the prophases, equatorial plate, anaphases, and telophases show an almost diagrammatic regularity in the arrangement of the chromatic granules. He explains the granular structure of the longitudinal halves of the chromosomes in the monaster by ascribing *the apparent loss of chromosomal continuity* to the same effect of fixing reagents which causes, in the earlier stages, the obliteration of the split in the chromatic filaments.

Strasburger (1884), working on Tradescantia, recognizes the Pfitzner granules as microsome disks and the *Zwischensubstanz* as hyaloplasm. He also claims, for the early prophase, that the material of the chromosomes may be in the form of a slender spiral. In his figures 63, 64, and 65 *a*, *b*, *d*, Plate XIV, he clearly shows the constricted surface outlines of the chromosomes in the metaphases, and in figure 49 of the same plate he shows it in the anaphases.

Miss King (1901), working on *Bufo lentiginosus*, finds the chromosomes of the ovum to be composed of microsomes.

Vejdovsky (1907) discusses the granular construction of the chromosome as described by Flemming. He says that young chromosomes do not consist of a homogeneous substance, but show a pale substratum on

whose surface are disposed fine granules separated at regular intervals. By treatment with the EH method, these granules acquire the deep black coloration of the earlier stages while the ground substance appears yet paler. According to him, the granules may be looked upon as chromomeres. On page 21 of the same paper he says that the *constricted chromosomes*, as they are figured by most authors, especially by Van Beneden, Herla, and Bonnevie, consist of independent knots or rings of chromatic substance in the ground material which corresponds to the original *Lininsubstanz*. The chromosomes appear here as if segmented, and the individual seg-

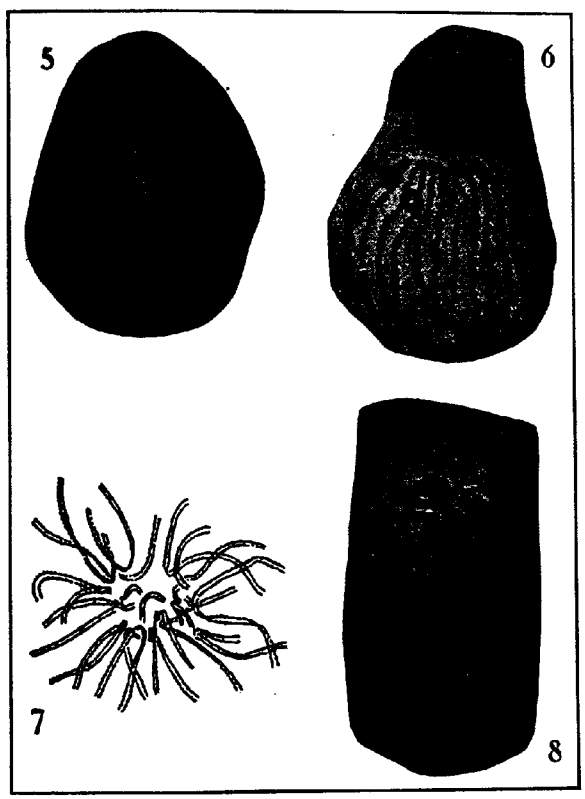

FIG. 5. Chromomeres figured in the metaphases by Flemming (1882). Salamandra. FIG. 6. Chromomeres in the prophases, figured by Hermann (1891). FIG. 7. Metaphase chromosomes of Salamandra showing granular composition, and assumed by Flemming (1882) to be longitudinally divided. FIG. 8. End views of chromosomes which show hollow and quadripartite structure. Merriman (1904).

ments of chromatic substance remind one of the chromioles of Eisen. Vejdovsky shows in his figure 164, *a*, *b*, *c*, *d*, *e*, *f*, and *g*, Plate IX, the metaphase chromomeres in Rhynchelmis and Enchytraeus. It is to be noted that this figure presents a striking resemblance to figure 38, *g*, Plate 3, of Wenrich's (1916) publication.

Vejdovsky says, further, that the slender halves of the split chromosomes consist of chromomeres bound together by delicate, slightly stainable bridges. He calls attention to the fact that the same structure was seen in the earlier stages of chromosome formation in Enchytraeus and assumes that, since they appear again later, their loss during the intermediate stages is due simply to the contraction of the chromosomes.

Later, in 1912, Vejdovsky describes the so-called *chromonema* which, in my opinion, is merely a very accurately described fixation distortion. His 1907 publication was, it seems to me, a more accurate presentation of the facts. One serious objection to the spiral structure was that it permitted no simple assumption as to the means for effecting the longitudinal splitting of the chromosomes. Vejdovsky avoids this difficulty by explaining, on page 21, that the spiral becomes altered, at metaphase, to groups of chromatic bodies in the nature of chromomeres which take up positions opposite one another so as to permit an equal longitudinal fission of the chromosome. (See his paper of 1912, Plate III, figures 42 and 43).

As I have already stated, I feel that Miss Merriman (1904) (text fig. 8) is not justified in extending her hypothesis of the quadripartite structure of the chromosomes to all the subdivisions that may occur, but it is interesting to note the persistence with which the structure shown by my text figure 2 is given by investigators. I refer to Bonnevie (1908), figures 7, 11, 19, and especially 22, Plate XI (text fig. 9), and in particular, to those of Nawaschin (1911) (text fig. 10), and Chambers (1915).

Von Herwerden (1910), working on Chironomus, believes the chromosome structure is spiral, but, on account of the granules she observed on them, she concludes by associating the latter with the ids of Weismann.

Schustow (1913) in his figures 4 and 6, Plate XIV (text fig. 11), and figures 29–33, Plate XV, shows the anaphase chromosomes to be hollow structures. Compare these figures with those of Bonnevie's (1908) figures 8, 10, and 22, Plate XI; 56, Plate XIII; and 75 and 79, Plate XIV. Although these figures of Bonnevie and Schustow do not especially show the chromomeres, they do illustrate the fact that the stainable material of the chromosome is peripherally placed, at least at these stages. The assertion of Schustow that the cross section of the early anaphase chromosome is circular whereas that of the late anaphase is polygonal has, I believe, no significance with reference to a telophase longitudinal split.

In discussing the distribution of the chromatin in the chromosome, Carnoy (1885) says:

> A l'intérieur de ce tube se trouve la nucléine, ou chromatine des auteurs. La manière

dont celle-ci s'y présente est variable. Ici elle remplit entièrement son étui; là, dans les boyaux volumineux, elle se retire contre la paroi, en laissant ouvert un canal central renfermant un plasma transparent.

Terni (1914) has, in many figures, very diagrammatic chromomeres. I consider his splitting chromosomes to be a misinterpretation of a chromatically hollow structure.

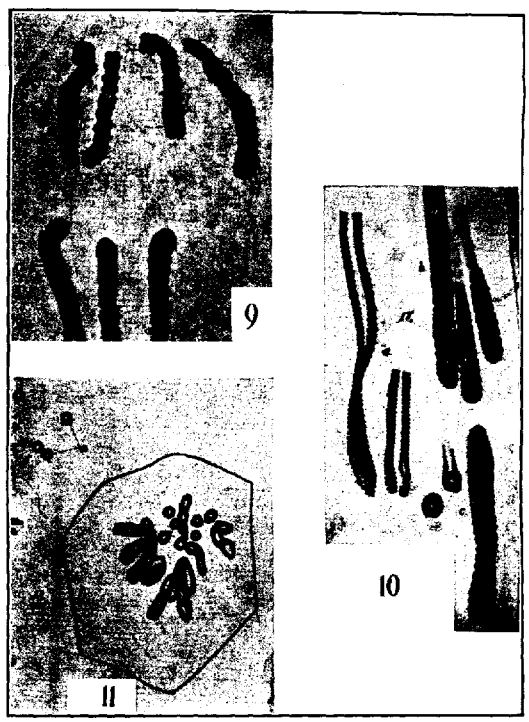

FIG. 9. Quadripartite chromosomes of Ascaris. Bonnevie (1908). FIG. 10. Chromosome end views showing quadripartite structure and chromomeres. Nawaschin (1911). FIG. 11. Chromosome end views showing chromatin-free centers. Schustow (1913).

Chambers (1915) working on *Disosteira*, describes a hollow chromosome structure in the living condition. He finds that the chromomeres are arranged in such a manner that there results a central achromatic core.

Wenrich (1916, p. 117) says, in speaking of Vejdovsky's 1912 publication:

I find little evidence of a *chromonema* in the telophase of the spermogonial divisions and what evidence there is would indicate that the chromatin becomes distributed on the inner surface of the vesicular walls, not on the outer surface of an achromatic core.

There is an implication here that the chromosome is hollow in somewhat the same sense as I have described it.

According to Suessenguth (1921), who worked on Rhoeo, one of the Commelinaceae, the chromosome has a moniliform structure made up of ten segments. Each of the latter, I take it, would be homologous to the chromomere as defined by Eisen. Eisen considered that the chromomere is made up of six chromioles, but that the number of chromomeres in a chromosome may be variable.

In conclusion, it seems to me, both from my own work and from the evidence in the literature, that the distinction between chromatin and linin is universally valid, and that, when preparations do not show this distinction, as in those of Grégoire and Wygaerts (1903) and of Sharp (1913, 1920) for the telophases, where, according to them, the chromosomes break up by vacuolation, it is due either to overstaining or to shrinkage.

The linin of the chromosome is distinctly a more or less regular cylinder of jelly-like consistency in which the chromatin is imbedded. It is not provided with an outer membrane which could be demonstrated by any of the methods used.

The chromatin is grouped into bodies, the generally recognized chromomeres. In Tradescantia these are of variable shapes and sizes, and, as far as present evidence goes, their number in the chromosome seems variable.

The colloidal structure of the chromosome is conceived to be primarily a two-phase system with the chromatin representing the disperse phase and the linin, the continuous phase. The continuous phase, within the body of the chromomere, may be more dehydrated than that present in the linin proper. That there may be still further phases of colloidal dispersion within the chromomere is not denied. At any particular stage of mitosis, an individual chromomere may have a greater or a lesser degree of dehydration than its neighbors in the same or in other chromosomes. This *degree of dehydration* may represent a specific constant for each chromomere for any particular stage. Since the coefficient of dehydration between the chromomeres may be a variable, only identical phases would be comparable, and it might be expected that in the *end result* of fixation, imbedding, and staining, the relative proportional dehydration values would be maintained.

The writer is indebted to Professor R. A. Harper and to Professor C. C. Curtis for many helpful suggestions during the course of this work.

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EXPLANATION OF FIGURES

PLATE XXIX

All the figures of this plate are from the chromosomes of *Tradescantia virginica* L. Figures 1, 2, 3, 4, 5, 7, 8, and 12 are freehand. Figures 6, 9, 10, 11, and 13 are drawn with the camera lucida at table height (1600 diameters), with 2 mm. Zeiss apochromatic objective N.A. 1.4 with 10x compensation ocular, at 160 mm. tube length and with critical illumination.

FIG. 1. Portion of a metaphase chromosome teased from a partially injured cell into the suspension fluid.

FIG. 2. Portion of a metaphase chromosome from the same cell.

FIG. 3. Enlarged end views of two separate chromosomes from different cells.

FIG. 4. Enlarged end view of another chromosome.

FIG. 5. Chromosome *a*, figure 6. The numbers are given for convenience in referring to individual chromomeres.

FIG. 6. Chromosomes of a pollen mother cell in the homoeotypic division. The lower dotted line represents the outline of the nucleus belonging to the other sister spindle which completed its dispireme in advance of the one drawn.

FIG. 7. A chromosome of the equatorial plate, first division, teased from the same cell as were those of figures 1 and 2. It was slightly damaged in the process, and I ascribe the constriction to the teasing. The upper figure shows the appearance at the top focus; the lower one is from the deeper focus.

FIG. 8. Chromosomes of the equatorial plate, first division, showing subdivision of the chromomeres. Taken from a perfect cell and a common figure.

FIG. 9. Metaphase chromosomes of the first division.

FIG. 10. Metaphase chromosomes with marked nodular outlines.

FIG. 11. Late prophase or diakinesis just prior to the equatorial plate stage.

FIG. 12. Chromosome from the same cell as those shown in figures 1, 2, and 7.

FIG. 13. Chromosomes in diakinesis.

PLATE XXX

The photographs were taken with a Zeiss apparatus, 2 mm. apochromatic objective, N.A. 1.4, compensation ocular no. 4, bellows 50 cm. Arc and color screens. × 1500.

FIG. 14. Metaphase (somatic) in the staminate hair of Tradescantia stained by aceto-carmine 1 : 4. The chromomeres are quite distinguishable.

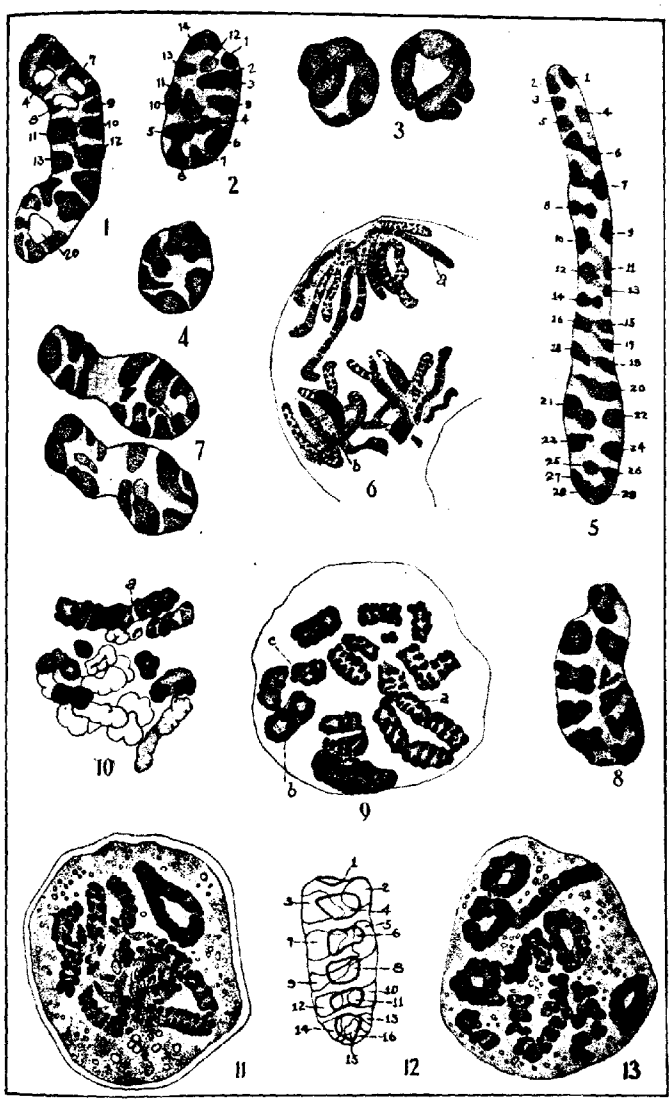
FIG. 15. The same stage in a staminate hair suspended in 3 percent cane sugar. Living chromosomes.

FIG. 16. An eight-hour preparation of metaphase pollen mother cells stained by 1 : 1 aceto-carmine. The chromomeres are especially clear. *Tradescantia virginica.*

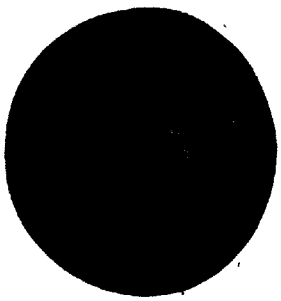
FIG. 17. Similar to figure 16, but showing the hollow construction of the upper chromosome as well as the spiral arrangement of the chromomeres in the linin matrix. *Tradescantia virginica.*

FIG. 18. Metaphase pollen mother cells of Rhoeo suspended in 1 : 1 aceto-carmine. Compare the serial order of the chromomeres with those of figure 17 and figure 16.

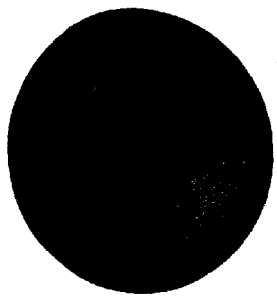
FIG. 19. Anaphase figure in the staminate hair of Tradescantia. Living chromosomes, intended to show the hollow chromosome structure in the two end views in the upper half of the division figure. Suspended in 3 percent cane sugar.



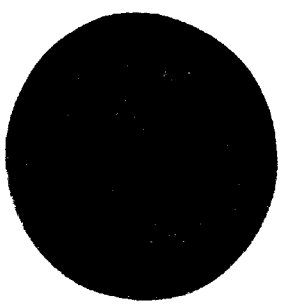
SANDS: STRUCTURE OF CHROMOSOMES



14



15



16

17

18

19

THE EFFECT OF RINGING A STEM ON THE UPWARD TRANSFER OF NITROGEN AND ASH CONSTITUENTS

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From a variety of experiments conducted to determine the effect of ringing on the upward transfer of carbohydrates (Curtis, 1920), evidence was obtained indicating that these foods are carried up the stem chiefly through the phloem tissues even though much of the food to be transported is present as sugar or starch in the xylem tissues. At least the upward transfer of sugars seems to be very clearly checked by the removal of a ring of phloem. When it was realized that sugar may be present in the vessels of the xylem at the time water is being carried in these tissues and yet may not be carried past a region where a ring of phloem is removed, the question immediately presented itself as to whether the commonly accepted idea that nutrients are carried in the "transpiration stream" is a correct one.

Though the literature is full of statements to the effect that inorganic materials absorbed from the soil are carried through the xylem in the "transpiration stream," these for the most part are based on mere assumption or on evidence which is far from conclusive. Certain facts have commonly been offered as proof of rapid transfer in the "transpiration stream." One of the commonest of these is that salts are dissolved in the soil water, and, as the water is known to be carried more or less rapidly through the xylem to the transpiring tissues, at first thought it would seem perfectly logical that the nutrients dissolved in the water would be absorbed and carried with it. If this were true, it would readily account for the rapid absorption and distribution of salts, but since the movement of water and that of solutes through osmotic membranes may be independent of each other, the salts are not necessarily carried with the water. Evidence showing that there is no direct relation between water absorption and salt absorption has been presented in the work of Muenscher (1922) and others. Whether or not the movement of water after absorption influences the transfer of solutes depends in part on whether the solutes get into the water-conducting tissues, and in part on whether these tissues form continuous open tubes with few or no obstructions. It would also depend on the method of water movement, that is, whether the movement is by mass flow throughout, or by diffusion throughout, or, as is perhaps more probable, by mass flow through certain regions and diffusion through others. The possible presence of associated parenchymatous cells, with-

drawing solutes from, or secreting them into, the vessels carrying the water, might also influence their movement.

The fact that solutes have actually been found in the water-conducting vessels and that they have been obtained from cut and bleeding stems has been offered as proof that they are carried with the water. As just stated, however, unless it is known that the water moves by mass flow through open tubes and not by diffusion or through occasional membranes, or unless it is known that neighboring cells do not remove solutes from a passing stream, perhaps transferring them and reintroducing them at a lower level, this can not be considered as conclusive evidence. Furthermore, the method of flow or bleeding from a cut stem may be different from that in a normal uninjured stem in the same sense that a movement of water through a series of cells with membranes enclosing solutions may be very different from the flow that would occur if some of the membranes were cut open.

The fact that, when cut stems are placed in solutions of various dyes, the solutes can be found at considerable distances in the xylem tissues has been considered as proof that solutes must be carried with the water. Normally, however, the water-conducting system may be considered as a closed system with no actual openings. The objections offered to the evidence from cut and bleeding stems should, therefore, be considered in this type of experiment also. Experiments involving the injection of dyes or other solutes through incisions of one type or another might be invalid for the same reason.

A few experiments have been reported in which the movements of dyes or of salts, such as those of lithium or iron, have been studied in rooted and uninjured plants, and some of these indicate a movement, or in some cases an accumulation, in the xylem; but, because of the toxicity of the materials used or of their tendency to be taken up by thick cell walls, or, as with lithium, the ease with which it penetrates membranes and the lack of quantitative determinations, these experiments have not yet given clear indications as to the method of movement or as to the tissues concerned in the movement of nutrient salts.

Since nitrogen is so commonly deficient in soils, and since the absorption of nitrogen can be relatively easily determined both quantitatively by analysis and qualitatively by the color of the plant and by its texture and growth responses, experiments were planned to determine the effects of ringing on the upward movement of nitrogen in stems. In a number of cases the ash contents of the leaves were also determined.

In most of the ringing experiments, twigs or branches were selected in pairs so that the ringed and the normal, unringed branches were similar in size and position. The precaution was also taken to select a branch for ringing such that there were always branches below the ringed one, so that the roots and trunk below would be well supplied with food and their

absorptive ability would not be lost through death or starvation. All ring wounds were covered at the time of ringing with warm paraffin. This precaution was necessary, since otherwise the parts above the ring would often wither. Paraffin instead of vaseline or grafting wax was used, for in previous tests it was found that vaseline was often injurious to stems and that grafting wax was injurious or less easily applied. Immediately after ringing, nitrogen as sodium nitrate was added to the soil. In a few cases calcium nitrate, calcium chloride, and sodium chloride were also used.

In every case the areas of the leaves, as well as the dry weights, were determined. The areas were determined by the use of a planimeter, and readings were determined to tenths of a square inch. With small leaves, like those of Ligustrum, it was found that the error in measurement was greater than that for the larger leaves. In most cases, each lot of leaves of known area was divided into halves or fourths for analysis, one of the halves or two of the fourths commonly being reserved for ash determination. Total nitrogen was determined by the Gunning method as modified to include nitrate nitrogen. The ash was obtained by combustion of the material in an electric furnace at a low red heat.

Since peach trees (*Prunus persica* Sieb. and Zucc.) distinctly show a deficiency of nitrogen by the formation of yellowish leaves and by their slow growth, and since they respond so readily to nitrate applications, experiments were first tried with these. In all the experiments with peach trees, branches one to three centimeters in diameter on young trees were selected. In ringing, bands of bark from one to two centimeters in width were removed.

In the first series, the branches were ringed in May just before the leaves were formed. At the time of collecting the leaves, those from the ringed branches were distinctly smaller; they were thick and stiff, yellowish in color tinged with red, and the younger leaves at the tip of the stem were very much curled. The check leaves from the unringed stem, on the other hand, were larger, thin and pliable, dark green, and not curled. By the end of the season the unringed branches had produced shoots several times as large as those of the ringed branches. For tree number 1, the three largest shoots developing from buds near the apex of the ringed branch averaged only 31 cm. in length. They had no side branches and only 50 leaves, while the three largest shoots on the unringed branch averaged 149 cm. in length. In addition, the check branches produced from 22 to 25 lateral twigs. These secondary twigs of the check were from 8 to 30 cm. long, and the total number of leaves was somewhat over 1,200.

From observations of the color and texture, it would appear as if the ring had checked growth by preventing the upward movement of nitrogen. It is possible, however, that the check in growth and the color of the leaves, as well as their texture, resulted from an excess of carbohydrates in those parts above the ring rather than from a deficiency of nitrogen. The results of analyses for the nitrogen and ash contents of these leaves are presented in table 1.

TABLE 1. *Effect of ringing on the nitrogen content of peach trees when the ring was made just as the buds were starting in the spring. Trees ringed May, 1920, and $NaNO_3$ added to the soil at this time*

Number of Tree	Branch	Date of Sampling	Number of Leaves	Area (sq. dec.)	Dry Weight (g.)	Total Nitrogen (mg.)	Nitrogen per sq. dec.		Nitrogen per g. Dry Wt.		Total Ash (mg.)	Ash per sq. dec.		Ash per g.	
							mg.	Relative to Check	mg.	Relative to Check		mg.	Relative to Check	mg.	Relative to Check
1	Check	7/21	10	3.779	2.0544	88.2	23.3	1.00	42.9	1.00					
1	Ringed	7/21	10	3.389	2.4500	56.0	16.5	.708	22.9	.532					
	Check														
1	*a*	8/27	20	9.257	6.398	246.40	26.7		38.5		388.0	41.9		60.6	
1	*b*	8/27	20	9.900	6.592						436.0	44.0		66.1	
1	*c*	8/27	20	9.404	6.647	245.84	26.1		37.0		397.8	42.3		59.9	
1	*d*	8/27	20	9.578	6.7334	252.56	24.6		37.5		355.0	37.1		52.7	
1	*e*	8/27	20		6.2573	232.80			37.2		319.8			51.1	
1	*f*	8/27	20		6.3074	222.60			35.3		339.6			53.8	
Average							25.8	1.00	37.1	1.00		41.3	1.00	57.3	1.00
	Ringed														
1	*a*	8/27	20	5.495	4.266	96.88	17.6		22.7		169.4	30.8		38.6	
1	*b*	8/27	20	6.011	4.905						180.0	29.8		36.7	
1	*c*	8/27	20	5.663	4.230	100.24	17.7		21.2		193.4	34.2		40.9	
1	*d*	8/27	20	5.521	4.6646	99.40	18.0		21.3		171.2	31.0		36.6	
1	*e*	8/27	20		4.7188	101.36			21.5		166.0			35.2	
1	*f*	8/27	20		4.2273	100.80			23.8		176.6			41.8	
Average							17.7	.689	22.1	.596		31.5	.761	38.3	.668

TABLE 1. *Continued.*

Number of Tree	Branch	Date of Sampling	Number of Leaves	Area (sq. dec.)	Dry Weight (g.)	Total Nitrogen (mg.)	Nitrogen per sq dec.		Nitrogen per g. Dry Wt.		Total Ash (mg.)	Ash per sq. dec.		Ash per g.	
							mg.	Relative to Check	mg.	Relative to Check		mg.	Relative to Check	mg.	Relative to Check
2	Check	7/19	10	4.547	2.8461	113.12	24.9	1.00	39.7	1.00					
2	Ringed	7/19	10	2.831	1.8749	46.62	16.5	.662	24.9	.626					
2	Check	7/21	10	4.063	2.357	95.90	23.6	1.00	40.7	1.00					
2	Ringed	7/21	10	2.728	1.8094	43.82	16.1	.680	24.2	.595					
	Check														
3	*a*	8/12	10	5.031	3.2160	122.36	24.3		38.0		239.8	47.6		74.5	
3	*b*	8/12	10	4.515	2.8216	97.44	21.6		34.5		180.2	39.9		63.8	
Average							22.9	1.00	36.2	1.00		43.8	1.00	69.1	1.00
	Ringed														
3	*a*	8/12	10	3.909	2.9540	65.62	16.8		22.2		*151.0	*38.8		51.1	
3	*b*	8/12	10	4.147	3.3693	68.6	16.5		20.4		123.8	29.8		36.7	
Average							16.6	.726	21.3	.586		34.3	.785	43.9	.499
	Check														
4	*a*	8/12	10	4.889	2.9634	107.24	21.9		36.2		165.2	33.8		55.7	
4	*b*	8/12	10	5.063	3.1846	110.04	21.7		34.5		221.2	43.6		69.4	
Average							21.8	1.00	35.3	1.00		38.7	1.00	62.6	1.00
	Ringed														
4	*a*	8/12	10	3.831	3.0043	71.12	18.6		23.7		*140.2	*36.6		*46.3	
4	*b*	8/12	10	3.515	2.8295	60.76	17.3		21.5		*125.0	*35.5		*44.1	
Average							17.9	.821	22.6	.638		36.1	.932	45.2	.720

* When the ash was taken out of these crucibles, it was found that some carbon still remained. This would explain the high readings.

It is evident from the data on the nitrogen content that ringing has resulted in a lessened amount of nitrogen in the leaves whether expressed as milligrams of nitrogen per leaf, per gram of dry matter, or per unit of leaf area. The small amount of nitrogen per leaf and per unit of weight might be expected, because the leaves are smaller and the dry weights are relatively high because removal of the carbohydrate has been retarded by the ring. The small amount of nitrogen per unit of leaf area, however, is a good indication that relatively less had been carried up the ringed stem, for otherwise one would expect more nitrogen as the leaves are thicker and heavier per unit of area. Furthermore, there was nothing to prevent the removal of proteins or other forms of nitrogen from the check leaves back to the trunk or roots, while such removal from the other leaves would have been retarded by the ring. The total amount of nitrogen moving up the unringed stem was certainly much greater than that moving up the ringed stem, for there were from ten to twenty times as many leaves on the check stem, and these leaves were also larger, usually from 30 to 70 percent, than those of the ringed stem.

Though nitrates have been found in abundance in stems (Berthelot and André, 1886), and organic nitrogen compounds have been found in solutions bleeding from cut stems (Schroeder, 1871), it is conceivable that much of the nitrogen might be transformed into protein which probably would be more readily carried in the phloem. In order, therefore, to determine if the rings had also retarded the upward movement of those nutrients which might be less likely to combine with organic matter and which have been found in abundance in the solutions bleeding from cut stems (Schroeder, 1871, and others), and which, therefore, are commonly considered to be carried in the "transpiration stream," the ash contents of a number of samples were determined. These data are also presented in table 1 for comparison with the nitrogen analyses.

In the most complete and dependable set of analyses, those for tree number 1, the leaves from the ringed stem have only 76 percent as much ash per unit of area as the checks, while per unit of dry weight they have only about 67 percent as much. The few analyses of samples from the other trees indicate similar differences.

Another experiment in some respects comparable with this one with peach trees was tried with a lilac bush. A stem three centimeters in diameter was ringed August 6, 1920. No leaf samples were taken at this time, however, and no nitrate was added to the soil. On July 30 of the following year (1921) other stems of this same bush were ringed and sodium nitrate was added to the soil. Leaf samples from the branch ringed the previous year, as well as from the newly ringed and check branches were taken at this time and again on August 22 and September 9. The shoot growth on the ringed stem was distinctly less than that on the check stem, the leaves were smaller, thicker, less pliable, and were distinctly yellowish

TABLE 2. *Effect of ringing on the nitrogen content of lilac leaves (Syringa vulgaris) when the ring is made the previous season. Stem ringed August 6, 1920. $NaNO_3$ added to soil July 30, 1921*

	Date of Sampling	Number of Leaves	Area (sq. dec.)	Dry Weight	Nitrogen Total (mg.)	Nitrogen per sq. dec.		Nitrogen per g.		Ash Total mg.	Ash per sq. dec.		Ash per g.	
						mg.	Relative to Check	mg.	Relative to Check		mg.	Relative to Check	mg.	Relative to Check
Check *a*	7/30	10	2.812	3.9463	97.72	34.8		24.8		282.8	100.5		71.7	
b	7/30	10	2.509	3.3832	85.40	34.0		25.2		248.2	98.9		73.3	
Average			2.711	3.6648	91.56	34.4	1.00	25.0	1.00	265.5	99.7	1.00	72.5	1.00
Ringed *a*	7/30	11	1.871	3.1778	43.96	23.5		13.8		111.0	59.3		34.9	
b	7/30	11	1.632	2.8111	40.32	24.7		14.3		96.2	58.9		34.2	
Average*			1.592	2.7222	42.14	24.1	.701	14.0	.560	94.2	59.1	.593	34.6	.477
Check *a*	8/22	10	2.864	4.148	112.56	39.3		27.1		382.8	133.6		92.2	
b	8/22	10	2.903	3.824	98.84	34.0		25.8		326.8	112.5		85.4	
Average			2.884	3.986	105.70	36.7	1.00	26.5	1.00	358.8	123.1	1.00	88.8	1.00
Ringed *a*	8/22	13	2.335	4.435	53.76	23.0		12.1		147.2	63.0		33.1	
b	8/22	13	2.780	4.787	59.60	21.4		12.5		160.8	57.8		33.5	
Average*			1.968	3.547	43.60	22.2	.606	12.3	.464	118.5	60.4	.491	33.3	.375
Check *a*	9/9	12	3.347	4.4344	112.84	33.7		25.4		352.0	105.1		79.3	
b	9/9	12	3.457	4.8689	127.40	36.9		26.2		380.6	110.0		78.1	
c	9/9	12	3.257	4.6588	123.78	38.0		26.6		342.8	105.2		73.5	
d	9/9	12	3.425	4.8406	129.36	37.9		26.7		350.4	102.3		72.3	
Average*			2.810	3.9173	103.20	36.6	1.00	26.2	1.00	297.0	105.7	1.00	75.8	1.00
Ringed *a*	9/9	16	2.515	4.4514	52.08	20.7		11.7		165.4	65.7		37.1	
b	9/9	16	2.457	4.3193	50.68	20.6		11.7		159.6	64.9		36.9	
c	9/9	16	2.670	4.7939	57.12	21.4		11.9		183.0	68.5		38.1	
d	9/9	16	2.496	4.9253	55.44	22.3		11.3		178.4	71.4		36.2	
Average*			1.584	2.8890	33.64	21.2	.580	11.7	.444	107.3	67.6	.640	37.1	.489

* Averages for ten leaves.

green in color with an abundance of red pigment. Analyses of the leaves from the branch that had been ringed the previous year and from the check branches are presented in table 2.

The data show that at the time of adding the nitrate (July 30), nearly a year after ringing, the check leaves had about 43 percent more nitrogen per unit of area than had the leaves of the ringed stem, that by August 22 they had 65 percent more, and by September 9, 73 percent more. On July 30 the check leaves had 1.79 times as much nitrogen per unit of dry weight as the ringed leaves, on August 22, 2.12 times, and on September 9, 2.24 times.

The ash analyses clearly indicate that the ring has hindered the movement of ash constituents also, the check leaves on July 30, August 22, and September 9 having respectively ash contents per unit of area of 1.64, 2.04, and 1.56 times that of the leaves from the ringed stem.

From these experiments with peach and lilac, it is very evident that the ringing has hindered the upward movement through the stems of both nitrogen and ash constituents. One could not conclude from these experiments alone, however, that the nitrogen or ash constituents move chiefly through the phloem instead of the xylem, because it might be true that they move up very largely through the newly formed xylem cells. The ring has not only cut the phloem, but has also prevented the formation of new xylem at the point of ringing. Furthermore, the changed growth above the ring might in some other way influence the nitrogen movement.

In order to eliminate some of these difficulties, another set of experiments was started after the shoots had become well developed and the leaves were mostly formed. Samples of leaves were taken at the time the rings were made and nitrate spread on the soil, and again later at different intervals. Because of an accident to the drying oven, most of the samples taken on the day of ringing were lost, and a second set was taken two days later but many of these also were later lost. These trees were growing in a poor, sandy soil, and at the beginning of the experiment the leaves were yellowish green and very characteristic of peach trees grown in soils deficient in nitrogen. A few weeks after applying the nitrate, the leaves on all excepting the ringed branches became darker green, and in many cases there seemed to be a renewal of shoot growth. The results of the analyses of this set are presented in table 3. For tree *C*, all the samples taken at the time of ringing were lost, except two, one of which consisted of the younger leaves from near the tips of several branches and the other of older, basal leaves. When samples were taken from this tree on October 7, the upper leaves were kept separate from the basal leaves. The young upper leaves from the ringed stem were very deficient in chlorophyll and were much curled.

It is evident from these results that, even after the new xylem and leaves have been developed, the cutting of the phloem has hindered the

movement of nitrogen into the leaves. The small number of samples collected at the beginning of the experiment has not allowed for any very accurate determinations of the percentages of increase. Yet the evidence is fairly clear that the ring has not entirely prevented the upward transfer of nitrogen, for the leaves above a ring show an increase per unit of area, but this increase is very much less than that in the check leaves. It is interesting to note that in the one instance where a narrow strip of phloem had grown over the ring, thus bridging the gap, the increase in nitrogen

TABLE 3. *Effect of ringing on the nitrogen content of peach leaves when the ring is made after the leaves have formed and the shoot growth is practically completed*

Tree	Branch	Treatment	Date Sampled	Number of Leaves	Area (sq. dec.)	Dry Wt. (g.)	Nitrogen Total (mg.)	Nitrogen per sq. dec.		Increase per Unit Area %	Nitrogen per g.	
								mg.	Rel.		mg.	Rel.
A	1′	Check	7/21	10	3.921	2.888	71.18	18.2	100		24.6	100
A	1	Ringed	7/19	10	4.469	3.330	84.42	18.9			25.4	
A	1	Ringed	7/21	10	4.592	2.993	80.64	17.6			26.9	
A	2	Ringed	7/21	9	3.921	2.433	66.78	17.0			27.4	
		Average						17.8	98		26.6	101
A	1′	Check (*a*)	8/12	10	4.431	3.207	116.76	26.4			36.4	
		(*b*)	8/12	10	4.560	3.156	114.66	25.1			36.4	
		Average						25.8	100	42%	36.4	100
A	1	*Ringed (*a*)	8/12	10	4.431	3.765	112.16	25.3			29.8	
		(*b*)	8/12	10	4.669	3.889	115.08	24.6			29.6	
		Average						*25.0	*97	*40%	29.7	*82
A	2	Ringed (*a*)	8/12	10	4.082	3.746	82.88	19.3			21.2	
		(*b*)	8/12	10	4.302	3.906	82.88	20.3			22.1	
		Average						19.8	77	11%	21.7	60
A	2′	Check (*a*)	8/27	20	9.011	6.757	246.12	27.3			36.4	
		(*b*)	8/27	20	8.753	6.720	241.36	27.6			35.9	
		Average						27.5	100	51%	36.2	100
A	2	Ringed (*a*)	8/27	20	7.637	8.591	164.08	21.5			19.1	
		(*b*)	8/27	20	7.982	8.435	159.60	19.9			18.9	
		Average						20.7	75	16%	19.0	53
B	1′	Check	7/21	10	4.696	3.065	96.18	20.5			31.4	
	2′	Check	7/21	10	4.460	2.836	90.30	20.2			31.8	
		Average						20.4	100		31.6	100
B	1	Ringed	7/21	10	4.651	2.875	81.48	17.5			28.3	
	3	Ringed	7/21	10	3.753	2.393	63.00	16.7			26.3	
		Average						17.1	84		27.3	86
B	3′	Check (*a*)	10/7	22	9.714	6.939	280.56	29.4			40.8	
		(*b*)	10/7	22	9.391	7.270	286.44	30.5			39.4	
		Average						29.9	100	46%	40.1	100
B	3	Ringed (*a*)	10/7	22	7.005	6.465	131.04	18.7			20.3	
		(*b*)	10/7	22	7.011	6.169	127.68	18.2			20.7	
		Average						18.5	62	8%	20.5	51

* A narrow strip of tissue had developed bridging the ring on this branch.

TABLE 3. *Continued.*

Tree	Branch	Treatment	Date Sampled	Number of Leaves	Area (sq. dec.)	Dry Wt. (g.)	Nitrogen Total (mg.)	Nitrogen per sq. dec.		Increase per Unit Area %	Nitrogen per g.	
								mg.	Rel.		mg.	Rel.
C		Leaves close to tip	7/21	10	3.477	2.010	73.78	21.2			36.7	
		Leaves close to base	7/21	10	5.328	3.140	100.38	18.8			32.0	
C_2		Upper leaves										
		Check (*a*)	10/7	10	4.199	3.5679	128.52	30.6			36.0	
C_2		(*b*)	10/7	10	3.857	3.2891	118.72	30.8			36.1	
C_2		(*c*)	10/7	10	3.670	3.0776	112.84	30.7			36.5	
C_2		(*d*)	10/7	10	4.076	3.3948	123.90	30.4			36.5	
C_2		(*e*)	10/7	10	4.283	3.7979	133.28	31.1			35.1	
		Average						30.7	100		36.0	100
		Upper leaves										
C_2		Ringed (*a*)	10/7	10	2.706	2.1400	30.24	13.7			14.1	
C_2		(*b*)	10/7	10	2.129	2.0087	27.58	13.0			13.7	
C_2		(*c*)	10/7	10	1.870	1.6834	23.94	12.8			14.2	
C_2		†(*d*)	10/7	10	4.644	4.9979	81.34	†17.5			†16.3	
		Average						13.2	43		14.0	39
		Lower leaves										
C_2'		Check (*a*)	10/7	10	5.263	3.623	115.92	22.0			32.0	
C_2		(*b*)	10/7	10	5.412	3.9376	130.76	24.2			33.2	
C_2		(*c*)	10/7	10	5.198	3.6550	101.08	19.4			27.7	
C_2		(*d*)	10/7	10	4.599	3.1287	96.04	20.9			30.7	
C_2		(*e*)	10/7	10	4.934	3.7445	133.00	27.0			35.5	
		Average						22.7	100		31.8	100
C_2		Ringed (*a*)	10/7	10	4.702	4.1740	75.64	16.1			18.1	
C_2		(*b*)	10/7	10	4.212	3.5381	62.44	14.8			17.7	
C_2		(*c*)	10/7	10	4.270	3.7556	64.12	15.0			17.1	
C_2		(*d*)	10/7	10	4.425	3.9574	70.56	15.9			17.8	
C_2		(*e*)	10/7	10	3.960	3.3688	61.04	15.4			18.1	
		Average						15.5	68		17.8	56

† These leaves were rolled like the others but were larger and more basal; not included in average.

above the ring was practically the same as that in the check. This ability of a narrow strip of phloem to allow for the transfer of an approximately normal quantity of nutrients is comparable to its ability to allow sufficient foods and nutrients to pass to insure approximately normal growth, as was previously found (Curtis, 1920).

A somewhat similar experiment was performed with a lilac bush (*Syringa vulgaris* L.). In this case paired twigs were selected, and one of each pair was ringed just above the fork. The rings were made on the current year's growth. It was hoped that it would be possible to analyze each twig

TABLE 4. *Syringa vulgaris. Effect of ringing on the upward transfer of nitrogen and ash constituents when the ring is made after the leaves are formed and shoot growth is completed. Ringed July 30, 1921*

	Date of Sampling	Number of Leaves	Area (sq. dec.)	Dry Weight	Nitrogen Total (mg.)	Nitrogen per sq. dec.		Nitrogen per g.		Ash Total (mg.)	Ash per sq. dec.		Ash per g.	
						mg.	Relative to Check	mg.	Relative to Check		mg.	Relative to Check	mg.	Relative to Check
Check *a*	7/30	10	3.335	4.5008	114.80	34.4		25.5		322.6	96.7		71.6	
b	7/30	10	3.315	4.1064	106.96	32.3		26.0		293.8	88.6		71.5	
c	7/30	11	3.586	4.6192	115.92	32.3		25.1		330.6	92.2		71.6	
Average*			3.303	4.2688	109.05	33.0	1.000	25.6	1.000	305.6	92.5	1.000	71.6	1.000
Ringed *a*	7/30	10	3.354	4.4176	115.36	34.4		26.1		307.6	91.7		69.6	
b	7/30	10	3.360	4.2288	110.32	32.8		26.1		320.2	95.3		75.7	
c	7/30	11	3.586	4.6338	117.96	32.9		25.5		332.6	92.7		71.8	
Average*			3.325	4.3196	110.97	33.4	1.011	25.9	1.013	310.1	93.2	1.008	72.4	1.011
Check *a*	8/22	10	3.322	4.6478	127.12	38.3		27.4		357.2	107.5		76.8	
b	8/22	10	3.264	4.6019	124.80	38.2		27.1		358.0	109.6		77.8	
c	8/22	10	3.231	4.5624	117.04	36.3		25.7		318.8	98.6		69.8	
Average			3.272	3.4530	122.99	37.6	1.000	26.7	1.000	344.7	105.2	1.000	74.8	1.000
Ringed *a*	8/22	10	3.709	6.0101	102.48	27.6		17.0		315.8	85.1		52.5	
b	8/22	10	3.496	6.4031	109.76	31.4		17.1		351.6	100.5		54.9	
c	8/22	10	3.670	5.8524	106.40	29.0		18.2		322.8	87.9		55.1	
Average			3.625	6.0885	106.21	29.3	0.771	17.5	0.653	330.1	89.2	0.848	54.2	0.725

* Averages for 10 leaves.

separately, but there were so few leaves on a twig and these were so variable in size that thirty leaves were taken from each treatment and these were divided into three samples each for analysis. The day after the nitrate was added heavy rains washed it into the soil. The analyses are presented in table 4.

The data indicate that the leaves from the check stem increased in nitrogen content per unit of area, while those from the ringed stem have slightly decreased. A similar gain by the check leaves and loss from the ringed is apparent in the ash analyses.

Similar experiments were performed in 1920 with *Acer platanoides* L., *Prunus pennsylvanica* L., and *Pyrus communis* L. In these cases the experiments were started in August, and there were no heavy rains to wash the nitrate into the soil until some time after it was added. The analyses of Prunus indicated a small increase of nitrogen in the check leaves and a still smaller increase in the leaves from the ringed stem, but the gain in the check was so small, only about 6 percent per unit of area, that the data are not presented. When expressed as percentage of dry weight, there was a distinct lowering in the leaves of the ringed stem, as would be expected. The analyses of the maple leaves picked 56 days after the nitrate was added to the soil showed no increase in nitrogen even in the check leaves. It would seem that all nitrogen absorbed had been taken up by other tissues before it could reach the leaves. This might be expected at this time of year, since quantities of carbohydrates must have accumulated in the various storage tissues. It is interesting to note that, though there was no change in nitrogen as measured per unit of area, the leaves from the ringed stems developed a very distinct purplish color, whereas the check leaves remained green. When the nitrogen content was expressed as percentage of dry weight, there was a distinct lowering in the leaves of the ringed stems.

From the data thus far presented, it seems evident that the ringing has reduced the upward transfer of nitrogen and ash constituents. It is conceivable, however, that the low content of nutrients in the leaves may be due, at least in part, to the retention of these constituents in the stem just above the ring, since these tissues have an excess of organic matter, the removal of which is prevented by the ring.

In order to test this point, an experiment was tried with California privet (*Ligustrum ovalifolium* Hassk.) in which pairs of uniform stems were used and both the leaves and the entire experimental stem were analyzed. In preliminary experiments it was found that the areas, weights, and nitrogen and ash contents of paired leaves of a given stem were very nearly equal. One from each pair of the more mature leaves of a given stem were removed at the beginning of the experiment, and the remaining leaves, together with the stems, were taken at the close. Three to six pairs of the younger leaves growing at the tops of the stems were left and

TABLE 5. *Effect of ringing on the nitrogen and ash contents of leaves and stems of Ligustrum. All stems ringed August 25 and harvested as indicated*

	Averages of 6 Stems						Averages of 6 Stems except as Indicated in Brackets						Averages of 7 Stems except as Indicated in Brackets					
	Check			Ringed			Check			Ringed			Check			Ringed		
e of Sampling ...	8/25	10/3	*Ave. Gain %	8/25	10/3	*Ave. Gain %	8/25	10/4	*Ave. Gain %	8/25	10/4	*Ave. Gain %	8/25	11/19	*Ave. Gain %	8/25	11/19	*Ave. Gain %
a of leaves														(6)	(6)		(6)	(6)
q. dec.).....	1.037	1.214	18.1	0.955	1.077	14.2	0.874	0.993	15.1	0.848	0.956	11.6	0.996	1.145	15.1	0.958	1.182	21.9
y wt. (g).....	1.2323	1.5168	25.2	1.1096	1.7115	56.7	1.0272	1.2356	20.6	0.9567 (5)	1.6805	75.6	1.0623 (6)	1.5164	45.1 (6)	1.0652 (4)	1.9869	8.3 (4)
rogen (mg.)..	17.69	40.41	139.0	15.59	18.99	26.2	15.17	28.61	92.8	12.6 (5)	15.77	19.1	16.43 (6)	40.11	171.3 (5)	15.40 (4)	13.57 (6)	4.8 (4)
rogen per sq. lec. (mg.).....	16.58	38.32	100.6	15.82	17.27	10.3	17.55	29.36	64.2	16.45 (5)	16.38	2.6	16.25 (6)	36.40	139.4 (6)	16.30 (4)	13.21	9.4 (4)
trogen per g. mg.).....	14.10	26.37	86.4	13.82	10.87	−20.6	14.72	23.24	48.7	13.97	9.38	−30.4	17.70	26.45 (5)	83.4 (5)	11.18	7.20 (5)	−47.1 (5)
h (mg.).....	93.5	164.3	73.4	82.4	98.7	23.4	77.3	115.4	48.7	77.1	91.6	18.0	76.3	126.4 (5)	97.6 (4)	76.1	85.0 (4)	16.4 (4)
h per sq. dec. (mg.).....	92.7	136.6	47.8	88.3	94.1	6.8	91.3	117.7	29.7	95.5	96.0	0.8	80.8	124.7	66.5	77.0	82.6 (5)	6.1 (5)
h per g. (mg.).	79.1	108.7	38.4	77.9	59.4	−21.4	76.5	94.1	23.2	81.7	54.8	−32.9	72.3	92.2	32.3	72.7	45.2	−39.3
y weight upper leaves ..														0.8846			0.9159	
trogen per g. upper leaves (mg.).....														25.04			8.58	
sh per g. upper leaves (mg.)...														75.7			36.3	
ry wt. stem, basal 20 cm. ..		2.0804			2.1933			1.7960			1.8697			1.9853			1.9059	
ry wt. stem, upper part....		1.6031			1.7674			1.5154			1.4410			2.1628			2.3123	
itrogen per g. stem, basal 20 cm.....		7.518			4.025			5.14			3.54			9.27			6.69	
itrogen per g. stem, upper part.....		8.673			3.932			6.65			3.52			10.40			(5) 6.87	

* These are averages of the gains for each stem and not the gain of the averages.

analyzed separately at the close of the experiment. Forty-two stems were selected for the experiment. These were chosen in pairs as they grew on the bushes of a hedge, and one of each pair was ringed while the other was left as a check. Sodium nitrate together with smaller amounts of calcium chloride, sodium chloride, and calcium carbonate were added to the soil around the hedge. The application was not very uniform because the bushes were growing close together and the bunches of sod beneath the bushes made uniform application difficult. This probably accounts very largely for the difference between the series taken October 3 and that taken October 4, since the two series were from different parts of the hedge. The data are presented in table 5.

From these data it is evident that there was a distinctly smaller increase of nitrogen in the leaves of the ringed stems whether this is considered as an increase in absolute quantity of nitrogen, or in the amount per unit of leaf surface. The amount of nitrogen per unit of dry weight shows, in every case, a loss in the leaves of the ringed stems. This was due very largely, of course, to the marked increase in dry weight of those leaves.

The data for the stems show that there is a greater amount of nitrogen per gram of twig in the checks than in the ringed stems. This is true in both the basal and the upper parts of the stem. The smaller amounts in the ringed stems are not merely apparent and due to the greater dry weights of the ringed stems which resulted from an accumulation of food, for, in each of the nineteen pairs of twigs analyzed in the three series, the check stem had not only a greater amount of nitrogen per unit of weight, but it had, with but two exceptions, a greater absolute amount of nitrogen than had the corresponding ringed stem. In one of these cases the basal part of the check stem had an unusually low amount of nitrogen, so low that it was evidently due to an error in analysis, and in the other case the ringed stem was not well matched with the check, but was distinctly larger, which fact would easily account for the greater total nitrogen.

In the series harvested Nov. 19, the wood and bark of the twigs were analyzed separately. The results of these determinations are given in table 6. The data for the leaves and for the entire twigs are included in table 5.

It is evident from table 6 that the ringing has resulted in a distinct increase in the weight of the bark, especially in the twenty centimeters immediately above the ring. This effect on the bark is even more clearly indicated by the increase in the ratio bark: wood, both in the basal and in the upper parts of the stem. The ratio between the amount of nitrogen in the bark and that in the wood is also increased by ringing.

The ash contents of the stems were not determined because of the small amount of material and the difficulty of dividing the samples, but those of the leaves were obtained and are presented in table 5 for comparison with the nitrogen. The results of the ash determinations correspond

very closely to those of the nitrogen determinations. There was a smaller absolute increase in the ash of the leaves from the ringed stems as well as a smaller increase per unit of area and a distinct decrease per unit of weight.

TABLE 6. *The effect of ringing on the nitrogen content of the wood and bark of stems. Averages of seven determinations except where indicated in brackets*

	Check Basal 20 cm.		Ringed Basal 20 cm.		Ave. Ratio Ringed / Check	Check Upper Part		Ringed Upper Part		Ave. Ratio Ringed / Check
		Ave. Ratio Bark / Wood		Ave. Ratio Bark / Wood			Ave. Ratio Bark / Wood		Ave. Ratio Bark / Wood	
Ave. dry wt. wood (g.)	1.4366		1.2217		0.827	(6) 1.3619		(5) 1.3465		(5) 0.943
Ave. dry wt. bark (g.)	0.5467	0.391	0.6842	0.606	1.240	(6) 0.8009	(6) 0.647	(5) 0.9658	(5) 0.819	(5) 1.241
Ave. total nitrogen, wood (mg.)	10.16		5.16		0.462	(6) 10.34		(5) 5.85		(5) 0.457
Ave. total nitrogen, bark (mg.)	8.34	0.854	8.05	1.904	0.915	(6) 12.37	(6) 1.387	(4) 12.58	(4) 2.669	(4) 0.901
Ave. nitrogen per g., wood (mg.)	7.13		3.90		0.545	(6) 7.33		(5) 3.38		(5) 0.499
Ave. nitrogen per g., bark (mg.)	16.11	2.25	11.41	3.15	0.738	(6) 15.43	(6) 2.17	(4) 11.09	(4) 3.14	(4) 0.747
Ave. nitrogen per g. stem (mg.)	9.27		6.69		0.731	10.40		(5) 6.87		(5) 0.682

The data thus far presented clearly indicate that ringing commonly hinders the movement of nitrogen and ash constituents into the tissues above the ring. This cannot be considered as conclusive evidence, however, that the phloem rather than the xylem is chiefly concerned in the movement of these nutrients. I have obtained considerable evidence showing that transpiration from the leaves of the ringed stems is distinctly less than that from normal leaves. This is due, in part at least, to the high concentration of solutes above the ring with a corresponding lowering of vapor pressure, and perhaps also to increased retention by colloids or to morphological or other changes. If the nutrients are carried in the so-called "transpiration stream," and if their rate of movement is determined by the water movement, a check in the transpiration from tissues above a ring would very probably reduce the movement of the solutes into these tissues. In order to eliminate this factor of transpiration, twigs of privet were experimented with as described below.

Twelve sets of three stems each were selected for the experiment. In every twig the young growing shoot was removed and four pairs of leaves were left at the apex. The four pairs of leaves immediately below these

were removed, leaving the twig bare as indicated in figure 1. On one twig from each set (no. 1) a ring was made immediately below the upper leaves. On a second twig (no. 2) in each set two rings were made, one just below the upper leaves and a second at the base of the defoliated part. The third twig (no. 3) was ringed at the base of the defoliated part and not at the point just below the upper leaves. Sodium nitrate was added to the soil around

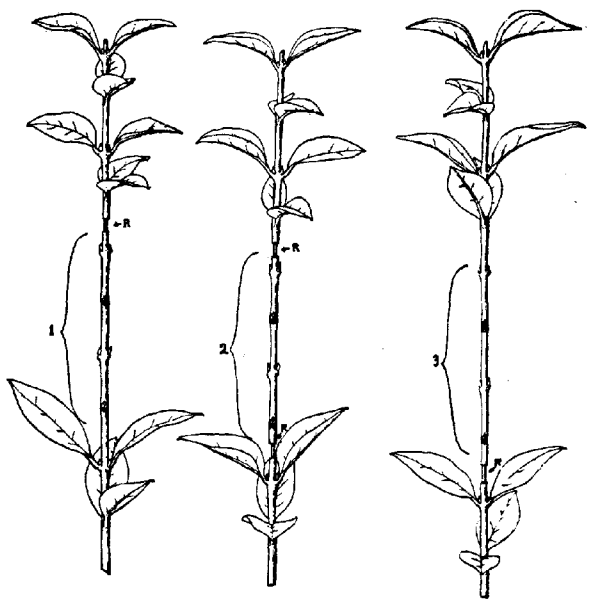

FIG. 1. Showing method of ringing so that the amount of water passing through the defoliated part of each stem would be approximately the same whether or not this part is isolated from the roots by a ring. 1. Check, medium carbyhodrate content in defoliated part. 2. Ringed, low carbohydrate content in defoliated part. 3. Ringed, high carbohydrate content in defoliated part.

the bushes at the time of ringing, August 25, and the twigs were cut for analysis on October 3. An analysis was made of the defoliated part of each stem as indicated by brackets in the figure. It was assumed that the amount of water moving through the stem would be approximately the same in numbers 1 and 2, since both had the same number of leaves on the stem at the apex and both were ringed immediately below these leaves. Since the defoliated part of the stem in no. 1 would probably have a higher sugar content than number 2, as it was not isolated by a ring from the leaves at the base, and since any such difference in carbohydrate content might distinctly alter its tendency to retain nitrogen, the third twig

TABLE 7. *Effect of ringing on the nitrogen contents of twigs when transpiration from ringed and check stems is approximately equal and where the carbohydrate content is low in one treatment and high in another. The ratios are averages of the ratios for each set, not ratios of averages**

Treatment	No.	Wood										Bark						Entire Stem	
		Dry Weight		Total Nitrogen		Nitrogen per g.		Volume		Nitrogen per cc.		Dry Weight		Total Nitrogen		Nitrogen per g.		Nitrogen per g.	
		g.	Rel. to Check	mg.	Rel. to Check	mg.	Rel. to Check	cc.	Rel. to Check	mg.	Rel. to Check	g.	Rel. to Check	mg.	Rel. to Check	mg.	Rel. to Check	mg.	Rel. to Check
Check.....	1	.7858	1.00	5.20	1.00	6.730	1.00	10.616	1.00	.5326	1.00	†(.4795) .4586	1.000	†(6.91) 6.63	1.00	†(14.492) 14.053	1.00	†(9.621) 9.497	1.00
Ringed, low carbohydrate	2	.6077	0.769	2.70	0.543	4.672	0.698	9.100	0.884	.3011	0.610	.3808	0.828	4.75	0.745 †(1.017)	12.533	0.889 †(0.728)	7.562	0.822 †(0.599)
Ringed, high carbohydrate	3	1.0797	1.616	3.99	0.723	3.219	0.419	12.938	1.328	.1280	0.455	.6242	1.497	6.76	1.135	10.778	0.776	6.078	0.633

* For no. 1 these are averages of 12 stems, except for volumes, which are averages of 11; no. 2, averages of 10 stems; no. 3, averages of 9 stems, except for volumes, which are averages of 8 stems.

† Including the ringed twig, which had healed over, in place of its check which showed an excessively low nitrogen content, evidently an error in analysis.

in each set was ringed only at the base of the defoliated part so as to allow the carbohydrate to move down from the upper leaves. Though no measurements were made, it was assumed that transpiration from these stems would approximate that from stems 1 and 2.

When the stems were cut they were tested with iodin to ascertain any evident differences in their starch contents. In the check (no. 1) starch was abundant in the xylem. In no. 2 no trace of starch could be detected, and in no. 3 starch was evidently somewhat more abundant than in no. 1. Averages of the data obtained are presented in table 7.

As indicated by the table, the bark and wood were analyzed separately. The volume of each twig was calculated from the length and the diameters at each end. Analyses of the wood showed that the ringing at the base of the defoliated part had resulted in a lower nitrogen content of that part whether measured as total nitrogen per stem, as nitrogen per gram of dry weight, or as nitrogen per unit of volume. This was true in each of the twelve sets with but two exceptions. One of the exceptions, in fact, adds weight to the evidence, for in this one twig the ring had healed over. The data for this one twig are not included in the averages. In the other exception, the difference was slight and probably due to inaccurately paired twigs. It should be mentioned in this connection that these twigs showed much greater individual variations than did the leaves. The nitrogen contents of the xylem, for example, expressed as percentages of dry weight, varied in the check twigs from a minimum of 4.21 percent to a maximum of 9.95 percent. For each set of three twigs, however, care was taken to select those similar in position, size, and color of leaves, and, with the exception just mentioned, the check in each set of three always had the higher nitrogen content.

The data also show that the effect of ringing on the nitrogen content of the wood above the ring is independent of its effect on the carbohydrate content. In one treatment (no. 2) the carbohydrate content was much reduced by the ring, as evidenced by the tests for starch and by the dry weights. In the other treatment (no. 3) it has increased the carbohydrate content as indicated by the same tests, yet in both cases the nitrogen content is distinctly reduced. In no. 2, the dry weight of the wood was reduced on the average by about 23 percent, the total nitrogen 46 percent, and the nitrogen per gram 30 percent. In no. 3, the dry weight was increased 62 percent, the total nitrogen was reduced 28 percent, and the nitrogen per gram was reduced 58 percent.

Analyses of the bark show that the rings at the bases of the defoliated stems resulted in a distinct reduction of the nitrogen in those which had a low carbohydrate content, but had practically no influence on those which had excess carbohydrate. The amount of nitrogen per gram of material seems to be clearly reduced. In two instances, not including the stem which had healed, there was a slightly higher amount of nitrogen

per gram of bark in the ringed stems with low carbohydrate (no. 2). This is probably in part accounted for by the distinctly lower dry weight of the bark of these stems, as there was no phloem connection with a carbohydrate supply. In no. 2, the dry weights of the bark samples were reduced on the average by 17 percent, the total nitrogen was reduced by 25 percent, and the nitrogen per gram by 11 percent. In no. 3, the dry weights of the bark were increased on the average by 50 percent, the total nitrogen was increased about 2 percent, and the nitrogen per gram was reduced by 22 percent.

Considering the twig as a whole, the rings at the bases of the defoliated parts distinctly reduced both their total nitrogen content as well as that per gram of dry weight.

Discussion

The results recorded in this paper are not in accord with those reported by Hibino (1917), who found that the proteins and ash contents of twigs of *Cornus controversa* were higher in the twigs ringed to the cambium than in the checks. Those ringed through the sapwood showed still higher protein, but lower ash than the checks. The protein contents in percentages of dry weight were 5.75, 6.31, and 7.81 respectively, in the check twig, in that ringed to the cambium, and in that ringed through the sapwood. The ash contents were, respectively, 1.45, 1.75, and 1.32. Evidently but very few twigs were analyzed, and since comparisons were made between different individuals, and since their composition was not known at the beginning of the experiment, the final differences may have been due to individual differences existing from the beginning. As discussed in connection with table 7, I have found the nitrogen contents of stems of privet to vary much more than those of leaves. Stems from similar plants were commonly found to vary in nitrogen content between 8 percent and 10 percent, and extremes were found with 6.39 percent and 14.96 percent nitrogen, a variation of over 100 percent.

When it was found that some nitrogen evidently passes a ring, as indicated in table 3, it seemed possible that on long standing a ringed stem might accumulate more nitrogen than a normal stem, since probably little or none of that passing a ring is ever carried back. The stems in Hibino's experiments had been ringed in July and were sampled in January. The data presented in table 2, however, indicate that leaves from a stem that had been ringed over a year may still have a lower nitrogen content.

Combes (1912) has reported ash analyses of leaves from ringed and normal stems of various woody plants. Expressing the ash as percentage of dry weight, the leaves from the ringed stem had lower ash contents in every case, and when expressed as percentage of fresh weight, all but two of the seventeen showed lower ash contents. When expressed as total ash or as ash per leaf, with the exception of *Pinus excelsa*, which in every

sample had more ash in the leaves of the ringed stems. there were no regular differences between the unringed and the ringed stems. As no leaf areas were reported, no definite conclusion can be drawn as to whether the ringing had altered the actual ash content.

It is very evident from the data presented in this paper that ringing has hindered the movement of nitrogen and ash constituents into the tissues above the ring. This has been found to occur in all the plants studied, including peach, cherry, lilac, and privet. Experiments were also tried with chestnut, maple, and pear, but these were done so late in the year that no increase of nitrogen was evident even in the check stems.

Whether the results can be considered as proof that nitrogen and other mineral nutrients move up through the phloem and not through the xylem with the water, may still be open to doubt. It is very probable that the rate of transpiration is usually less from the ringed stems. If there is an actual mass flow of water in the transpiration stream without passing through filtering membranes, a flow as through a pipe in an ordinary water system, if nutrients are carried in this stream and the associated living cells do not remove them, then the rate of transpiration would, of course, influence the amount of nutrients reaching the transpiring tissues. So far as I am aware, however, nothing as yet has been published showing conclusively that there is such a "transpiration stream" in plants or that the rate of transpiration directly determines the amount or rate of nutrient movement to the transpiring tissues. The one preliminary experiment reported in table 7 would indicate that transpiration is not an important factor directly determining the distribution of nutrients. The data presented by Muenscher (1922) give perhaps even better evidence that transpiration does not directly influence the distribution of nutrients. Though his experiments were chiefly directed to determine the influence of transpiration on the absorption of nutrients, they offer evidence that it does not influence nutrient movement after absorption, for, if the removal of nutrients from the roots were hastened by transpiration, this hastened removal should cause greater absorption for the plant as a whole and should reduce the ash in the roots, the absorbing organs. He found, on the contrary, that, when transpiration was altered by light and shade, plants with high transpiration rates showed an ash content of the roots distinctly higher than those with low transpiration, when measured as total ash or as percentage of dry or green weights, and an ash content of the tops somewhat lower in percentage but higher in total amount. Of course, in neither case do the ash contents indicate the amounts present in the conducting system. This high ash content in the roots is probably due to their increased content of organic matter, and, in fact, it is difficult to arrive at any definite conclusion with respect to the effect of transpiration on the distribution of nutrients in experiments of this type in which the organic content of the tissues is not under control.

Furthermore, even though transpiration above a ring may have been reduced, the high carbohydrate content of the tissues above the ring, as well as the ring itself, would tend to prevent the removal of nutrients once they got up past the ring. This failure in removal would tend to offset any possible slower absorption that might result from decreased transpiration.

Aside from difficulties in interpretation, resulting from possible differences in transpiration, there is the difficulty that the xylem itself may have been altered by the ringing. Several writers (Daniel, 1906; Combes, 1912; Higgins, 1919, and others) have offered evidence that tyloses or gum may plug the xylem vessels in the region of a ring or other wounds, but in none of those instances that have come to my attention have precautions been taken to protect the exposed xylem. In nearly every one of my own experiments in which the exposed xylem of small twigs has not been immediately covered with a layer of melted paraffin, the leaves above the ring have shown withering sooner or later. Those that were protected rarely showed withering within the time of the experiment, and in no case were samples taken from stems that showed withering. In other experiments, however, which are not yet completed, I have obtained indications that rings even when protected may increase the resistence of a stem to the flow of water. Even if it were conclusively demonstrated, however, that ringing resulted in a partial plugging of the xylem, it could not be said with assurance that such plugging is the reason for the lower nitrogen and ash contents in the tissues above a ring.

Summary

Experiments are reported showing the effects of ringing a stem on the upward transfer of nitrogen and ash constituents. Data from such experiments with privet, peach, and lilac are presented.

The data show that a ring distinctly hinders the movement of nitrogen and of ash constituents into the leaves above the ring, either when the ring is made in the spring before the leaves open and the new xylem is laid down, or when it is made in the summer after they have opened and the new xylem is partly or fully formed.

When sodium nitrate, with or without other nutrient salts, is added to the soil, the nitrogen and ash contents of the leaves from unringed stems increase to a much greater extent than do those of the leaves from ringed stems. This is true whether data are expressed per unit of dry weight, per unit of leaf surface, or as absolute quantities.

The stems also were analyzed to determine whether or not the low nitrogen content of the leaves could be accounted for by accumulation of nitrogen in the stems. These analyses, whether of the entire stem or of the wood and bark separately, showed a lesser content in the ringed stems.

A single experiment was tried in which an attempt was made to eliminate

the influence of altered transpiration or of change in carbohydrate content. In this experiment, evidence was obtained indicating that the low nitrogen content above a ring is not due to a lessened transpiration or to a changed carbohydrate content.

The data cannot be considered as conclusively proving that nitrogen or other nutrients move upward primarily through the phloem and not through the xylem, for the treatments may have altered the xylem tissues. Aside from such a contingency, however, these data from ringing experiments offer strong evidence indicating that nutrients are carried chiefly in the phloem.

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THE INFLUENCE OF PLANTS ON THE AIR IN HOUSES

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THE PROBLEM

The experimental work reported in this paper deals with the percentage of carbon dioxide in the air of a city greenhouse at various times of day. The discussion deals with the relation between the presence of plants in rooms at night and the comfort and safety of persons occupying the rooms.

It has been known [1] for several years that ventilation of occupied rooms is necessary to keep down the temperature, secure cooling movements of the air, and maintain a low humidity, and not to let out air containing a high percentage of carbon-dioxide and admit that having a low carbon-dioxide content. It is possible also that one good effect of ventilation is the removal of injurious organic matter[2], and it is certain that it is beneficial in preventing the continued multiplication of pathogenic organisms.[3]

In the light of this information, it seems strange that so little attention has been paid to the nature of any effects that the presence of plants may have on the air of rooms occupied for sleeping purposes. There is a widespread popular belief that plants in a room at night are injurious to persons sleeping in the room. The recent experience of one of us in a large hospital where an attendant is kept busy for several hours every day removing plants from the rooms at night and replacing them in the morning has directed attention especially to this belief.

While most textbooks on botany and physiology simply ignore the subject of the relation between plants and people in sleeping rooms, a widely used school chart bearing the statement that it has been revised by the dean of the college of agriculture in a tax-supported university says:

> We can understand why plants should have no place in sleeping rooms at night for when light is withdrawn, the absorption of carbon dioxide stops and the plants give off this poisonous gas.

Since Winslow [4] has found that air contaminated by the breathing of human beings until it contained an average of 20 to 60 parts of carbon-dioxide per ten thousand parts of air, five to fifteen times the normal amount,

[1] Winslow. Sci., n. ser., 41: 625–632. 1915.

[2] Since the above was written the writers have seen the Report of the New York State Commission on Ventilation (Dutton and Co., New York, 1923) which states (p.8) that the weight of scientific evidence is against the existence of organic poisons in respired air.

[3] *Cf.* Nolte. Ann. Mo. Bot. Gard. 1: 47–80. 1914.

[4] *Loc. cit*; also Rept. N. Y. State Com. on Ventilation cited above.

produced no bad effects on the occupants of the room so far as physiological and psychological tests show, and since Pettenkofer [5] long ago showed that carbon-dioxide is quite without effect on human beings in the highest concentration that it ever attains in occupied rooms, our attention has been directed especially to the percentage of carbon-dioxide, during the day and night, in a room containing a large number of growing plants.

The work was carried on in the greenhouse in Volunteer Park in Seattle. The room in which the samples of air for analysis were secured was well filled with plants, so that the total leaf area exposed was large in proportion to the volume of air. The investigation was carried on in July when the plants were growing actively. The room contained a considerable number of tropical plants whose growth was especially rapid and whose metabolism was thus very active. The plants were potted in rich soil fertilized with organic fertilizers every two weeks, and no doubt microorganisms in the soil were active agents in the production of carbon-dioxide. The room had top ventilation of the type commonly provided in greenhouses.

Experimental

Hesse's method as reported by Scott [6] was utilized for determining the carbon-dioxide content of the air. The necessary apparatus together with the standard solutions were taken to the greenhouse, where all analyses were performed. This eliminated the transport of samples to the laboratory and facilitated the checking of data. The results were calculated for dry air at standard conditions and are reported in the number of parts of carbon dioxide per ten thousand parts of air.

All precautions were taken to keep reagents and samples of air collected from contamination by carbon-dioxide. The samples were secured by emptying a large Erlenmeyer flask, previously calibrated, of distilled water saturated with the air to be sampled and having the same temperature. As soon as the flask was emptied, it was immediately corked and taken to the titration bench where standard barium hydroxide was added and the flask was shaken for ten or more minutes. The excess of alkali was titrated with standard oxalic acid, after washing down the sides of the flask with boiled distilled water. The solutions were admitted to the flask through a hole in the stopper of the flask. The data obtained are given in table 1.

Discussion

It is evident that certain factors tend to decrease the amount of carbon-dioxide in the air of this greenhouse, while others tend to increase it. Under the first head come (*a*) the green plants which use carbon-dioxide in photosynthesis during the day but not at night, and (*b*) ventilation, including

[5] Quoted by Winslow, *loc. cit.*

[6] Standard methods of chemical analysis, p. 728.

wind, which creates more or less draft through the room. Under the second head come (*a*) the presence of people, producing carbon-dioxide by their respiration, (*b*) the green plants likewise producing carbon-dioxide whether in light or in darkness as the result of respiration, and (*c*) the micro-organisms also producing carbon-dioxide at all times.

TABLE 1. *Parts per Ten Thousand of Carbon Dioxide in Air of a Greenhouse at Various Times of Day**

Time		Parts CO_2 in 10,000
1921		
July 12	4:10 P.M.	10
13	8:30 A.M.	6
13	3:10 P.M.	8
14	3:00 P.M.	5
15	10:30 A.M.	9
15	5:20 P.M.	5
16	12:50 P.M.	6
18	6:45 A.M.	4
18	7:00 P.M.	4
19	7:05 A.M.	5
19	7:00 P.M.	6
20	2:45 P.M.	4
21	6:45 P.M.	4
21	8:50 P.M.	3
22	7:00 A.M.	3
22	10:50 A.M.	4
22	6:15 P.M.	5
22	9:50 P.M.	5
23	1:30 P.M.	6
24	1:00 P.M.	5
24	7:45 P.M.	6
25	1:45 P.M.	6

* Calculated for dry air at standard conditions.

It might be expected that during the day photosynthesis, using carbon dioxide, and respiration, liberating it, would approximately neutralize each other, and that thus the plants would have little effect on the net amount of carbon dioxide in the air; while at night, photosynthesis being stopped and respiration continuing, the carbon-dioxide content of the air would be increased. On this basis, one should expect a greater percentage of this gas in the morning when the plants have been several hours in darkness than in the afternoon when they have been in light for some hours. The tests, however, show just the reverse of this. The average of the seven carbon-dioxide determinations made in the forenoon was between 4 and 5 parts per ten thousand, and that of the twenty in the afternoon, between 5 and 6 parts per ten thousand.

It does not seem probable that the activities of the soil organisms could account for this difference, though it is known, of course, that the rate of respiration in many plants is increased by intense illumination. Ventilation might either increase or decrease the carbon-dioxide content of the air in the greenhouse, but, since it was at the time of all tests higher than that of normal air, it is evident that it never increased but always

tended to decrease it. There seems to be no reason to suppose that ventilation was more effective in decreasing carbon-dioxide content at night than during the day.

The only remaining cause that could account for the accumulation of carbon-dioxide in the daytime was the presence of people visiting the greenhouse. The number of visitors varied greatly on different days and at different times of the day. A very high percentage of carbon-dioxide was found at one time when a gathering of florists and botanists inspected the greenhouse and it was crowded during the entire afternoon, and also on another occasion when a large number of school children came to see the plants. The irregularity in the number of visitors is undoubtedly the cause of the wide discrepancies in the values found at different times. The greenhouse was closed to visitors at 6 P.M.

Since, according to Winslow's data, the amount of carbon-dioxide could be increased to a point five to fifteen times as great as the maximum found in this greenhouse without injury to people, and since the amount of leaf area exposed here was very large in proportion to the volume of the air, while it is very small where a few plants are kept in sleeping rooms, it would seem that the amount of carbon-dioxide produced by a few plants in a sleeping room would not reach the danger point for the occupants or even approach it. Any danger must rest on other grounds, such as high temperature, high moisture content of the air, or some other factor.

Summary

1. Determinations of the carbon-dioxide content of the air in a greenhouse indicate that the concentration of this gas did not at the time of any determination reach a high enough concentration to be injurious to human beings.

2. Under the conditions existing in this greenhouse, the effect of plants in increasing carbon-dioxide content is negligible in comparison with the effects of the people who visit the greenhouse.

3. Any bad effects that may at any time have been experienced from the presence of plants in reasonably ventilated sleeping rooms must rest on some other basis than carbon-dioxide production.

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THE POTENTIALITIES OF A CELL[1]

CHARLES E. ALLEN

I

On the basis of evidence now at hand, it is generally agreed that the flagellates represent the ancestral group from which came most existing plant and animal phyla. More specifically, it is to the free-living flagellates that we look, and, as concerns most groups of plants, to the pigmented forms, for the nearest contemporary approach to phylogenetic origins. It is not to be forgotten that the flagellates now living represent the outcome of as long an evolutionary development in point of time from any common point of departure as do the angiosperms; and that only very cautiously may conclusions be based upon present-day flagellates as to the nature of the forms from which higher organisms have been derived. With the latter fact in mind, however, certain suggestions of a very general nature seem fairly safe.

One notable characteristic of the pigmented flagellates is a *plasticity* in form and function. The familiar Chlamydomonas, for example, may take the form of a flagellate cell; of an amoeboid cell which ingests organic food in an "animal-like" fashion; of a non-motile cell, divisions within whose wall result in the formation of a temporary colony; of a flagellate gamete; of a non-flagellate zygote, differing from the "vegetative" quiescent form in the nature of its wall and of its secretions; and of a palmelloid colony.

Myxochrisis paradoxa may be selected as another illustration. This brown flagellate, according to Pascher's description, appears as cells, single or in small groups, with rigid walls, one-, two-, or several-nucleate; as naked, one-nucleate flagellate cells; as amoeboid cells; as filar plasmodia formed by the partial union of amoebae; as "true" plasmodia, resulting either from the growth of single amoebae accompanied by nuclear division, from a fusion of several or many amoebae or plasmodia, or from a combination of the two processes; and as encysted plasmodia which by division form temporary colonies. In each phase, some individuals lack the characteristic brown chromatophores. Cell division seems to occur in any phase except possibly the amoeboid. Different phases are characterized by holophytic or holozoic nutrition, or by a combination of the two methods.

These instances appear to be fairly typical. Many species of both green and brown flagellates are described whose visible cellular organiza-

[1] Address of the retiring president of the Botanical Society of America, read at Boston, December 28, 1922.

tion is from time to time profoundly modified, the transition from one phase to another seeming to occur on relatively slight provocation. Such plasticity is especially striking in view of the present very imperfect knowledge of most species; indeed, it is not certain that all the phases into which any one species may pass have yet been recognized. A comparable plasticity, it may be observed, characterizes other protistan groups; recent reports, for example, indicate the occurrence in bacteria of comparable and quite remarkable modifications in cell size and cell structure. The pigmented flagellates are selected for present mention because of their apparent phylogenetic significance.

In entering upon and in passing through a particular phase (such as the amoeboid), the cell of a flagellate race performs certain functions. The ability to perform each of these functions results from the chemical and physical make-up of the living matter or of some of its parts. Since all known living matter is organized into cells, the possibilities or *potentialities* that inhere in the constitution of the living matter may be spoken of as the *potentialities of the cell.* Each function that the cell or one of its parts performs is the expression of a potentiality.

The expression of certain potentialities, particularly of those concerned in the more fundamental processes, is common to two, or to several, or to all phases. But the potentialities manifested in one phase differ, as a group, from those manifested in another phase. Smaller differences exist between different stages, for example, of the amoeboid phase, as to the particular group of potentialities momentarily functional, or as to the degree or manner of expression of particular potentialities. Some potentialities can be expressed only in conjunction with certain others; some are mutually exclusive; and from the latter fact arises the possibility of distinct phases in the history of the race. A list of the potentialities of any cell, therefore, would be in effect a list of all the functions,the capacity for which is inherent in the living matter of that cell.

A distinction may be drawn between an actual change in the form and activities of a cell—as the transition from a flagellate to an amoeboid form—and the origin from the cell, by division, of cells of a different form—as in the production from a plasmodium or a multinucleate cyst of numerous flagellate offspring. In either case, the *race* passes from one phase to another; the individual *cell* may not. Obviously a cell can transmit to its offspring only the potentialities which it possesses; and hence the potentialities of all phases possible to the race are possessed by any cell in any phase; even though the cell, in a particular phase, is itself debarred from expressing some of its inherent potentialities.

This fact, that a cell possesses potentialities which, having reached a certain phase, it can not itself express although it can transmit them, calls attention to the widely recognized dual constitution of living matter. The heritable potentialities arise from what may be called, non-commit-

tally, some *fundamental features* of cellular organization. The persistence of these fundamental features is consistent with marked alterations in the form, the internal structure, and the functions of the cell. The persistent, heritable features of organization are undoubtedly in large measure, in nucleated cells, especially characteristic of the nuclear substances, the cytoplasm being the medium through which from time to time varied sets of potentialities are expressed. However, the division of functions is probably not quite so sharp as this statement would imply; for some few, at least, of the hereditary potentialities of the cell seem to depend upon persistent features of the constitution either of certain parts of the cytoplasm, such as plastids, or of the cytoplasm as a whole.

The passage of the cell from phase to phase is conditioned by stimuli. This is true at least to the extent that, when a change is to take place, surrounding conditions determine just what that change shall be. Whether *some* change in the activities of the cell would occur if the environment remained unmodified, no one can say. Indeed, the activities of the cell necessarily modify its environment; so that an unchanging environment for a living cell is unthinkable. Experiment shows, however, that a very large proportion of the processes of change that constitute life are or can be brought about by environmental changes; and that, as between two or more possibilities at any point in the story, the environment largely determines which alternative shall prevail. To this extent, a particular potentiality may be described as the power of responding, by a certain activity, to a definite stimulus or group or class of stimuli. In recognition of this point of view, various writers have described all the characters of an organism as responses.

But this statement, like most generalizations, may be too broad. That the organization of the living matter not only establishes certain potentialities, but, in conjunction with the environment, plays a part in determining the sequence of expression of those potentialities, is suggested by various facts. One such fact, already cited, is that a cell, in any particular phase, can not pass indifferently into any other phase whose potentialities it possesses. Limitations of this nature result in the appearance of something approaching a life cycle. For instance, from the multinucleate sclerotium of Myxochrisis may come, after cell division, thick-walled quiescent cells, flagellate cells, or amoeboid cells; a quiescent cell, forsaking its wall, may become flagellate or amoeboid; and a flagellate cell may pass into the amoeboid phase. In spite of a considerable range of alternatives, the story moves in a certain general course; for an amoeboid cell does not, as a rule at least, reverse the order and take on the flagellate or the quiescent form. It is too early to say what further variations of the history unusual conditions may bring about; but a general tendency toward something like a cycle seems manifest. As there is a plasticity in form and function, so there is a plasticity in the sequence of forms and functions; but both

types of plasticity are measurably limited. The tendency toward a certain succession of phases sometimes, no doubt, results from limitations imposed by the conditions of the particular present phase. Thus, a plasmodium or a multinucleate cyst, of whatever species, can not, or at least usually does not, return to the uninucleate condition except by a division into uninucleate cells, in which process the identity of the mother cell disappears. Here a different course of events is clearly difficult or impossible. But the limitations noted upon the sequence of quiescent, flagellate, and amoeboid phases have a less obvious physical basis. Such limitations, like the potentialities whose expression they affect, are inherited. Like the potentialities, they seem to have their basis in the fundamental organization of the living matter.

Other heritable characters that may be considered directing or limiting tendencies are polarity and the various specific types of cellular symmetry. In the same category perhaps belong some of the "inhibiting" and "lethal" factors of genetic analysis. It appears, then, that the fundamental organization of living matter, besides giving rise to a great variety of potentialities, likewise in some degree limits and directs the order of expression of those potentialities. The question is still open whether the living matter, apart from the interaction of the environment, is able to do more than limit and direct—namely, to *initiate* the expression of any of its own potentialities.

Especially characteristic of living matter is one potentiality of far-reaching significance—that of undergoing changes in its fundamental features. From time to time, and under almost entirely unknown conditions, the specific character of the living matter is altered; some of its potentialities may be modified, some lost, or new ones acquired; and thus a new race is born. The possibility of changes in fundamental organization is limited; evolution can not move in any conceivable direction. The directions in which it may move are themselves determined by the constitution of the living matter. A question now arises paralleling that already asked concerning the expression of specific potentialities: How far are evolutionary changes themselves responses to stimuli, and how far the necessary result of the cell's constitution? To what extent, if at all, would new races arise if the relatively persistent features of the organization of living matter were uninfluenced by the environment? This question has been variously answered, but it is doubtful if any of the answers is more than an interesting guess.

II

Each of the types of evolutionary change just suggested—loss, gain, and modification of potentialities—is illustrated by flagellates that have become adapted to a strictly or mainly holozoic, saprophytic, or parasitic existence. Such highly developed parasites as the Trichonymphidae, for example, have lost the potentialities concerned in photosynthesis. Cor-

related with this loss, whether simultaneous or subsequent, was the disappearance of chromatophores. In other ways the range of potentialities has been limited, so that the cell can assume (so far as known, with the possible exception of one species) only the flagellate form. The plasticity of the pigmented species, as shown by the number of phases possible to the organism, has been reduced.

Some of the changes accompanying the complete loss of potentialities consist in the modification, rather than the loss, of certain powers of response. The specialization in adaptation to a parasitic mode of existence implies that the race remains under a greater variety of conditions in a phase suited only to parasitic nutrition. That there has been a real change in responsive power is shown by the great difficulty of keeping these organisms alive in any habitat other than that to which they are so narrowly adapted.

On the other hand, new potentialities have appeared. Without much doubt the adaptation to a parasitic existence has involved the acquisition of new powers of digesting and assimilating the types of nutrients now available. New potentialities certainly show themselves in the development of intracellular structures, notably the elaborate neuromotor apparatus recently described in detail by Kofoid and his students. It is quite possible, too, that the loss of the power of passing into certain phases is in a measure compensated for by an increased power of responding by relatively small modifications to the minor environmental changes which are all that an internal parasite ordinarily encounters during its active life.

Comparable changes have occurred in the evolution of other protists from flagellate ancestors. Each phase in the history of a slime mold such as Stemonitis, except that of the sporange—walled spore, swarm-spore, amoeba, microcyst, plasmodium, sclerotium—is duplicated, or simulated, in the life cycles of numerous flagellates. So many phases persist that, until the course of descent of the myxomycetes is known, it will be impossible to say what, if any, potentialities have been lost. On the other hand, new potentialities, leading to the formation of a rather complex sporange and capillitium, have been acquired. It is possible, however, that some of the potentialities concerned in sporange-formation are not really new to the myxomycetes, but are old ones which have been modified in time of appearance, in degree, or in duration. In large measure, too, there have been modifications in responsive power which affect the duration of the various phases.

The life cycle of Stemonitis consists of a series of phases, still with marked possibilities of substitution, suppression, and extension, but none the less following one another in fairly definite order and in the main incapable of inversion. The tendency operating in the pigmented flagellates toward some sort of sequence of stages seems to have become more effective. Thus the change in fundamental organization which has diminished the flagellate

plasticity involves, in the case of a slime mold, not so much a loss of particular potentialities as the organization of the expression of potentialities into a more nearly rigid series.

III

The changes involved in the evolution of coenobic species may be similarly outlined. No sharp line exists between temporary colonies which, for the period of their existence, are coenobes, and the more durable colonies that characterize some flagellates as well as many derived forms. So far as concerns the potentialities upon which the formation of a coenobe depends, perhaps little strictly new appears at this evolutionary level, because the prototype of each method of colony-formation (instance those of Scenedesmus, Hydrodictyon, Tetraspora, and Spirogyra) is to be found among the flagellates. Changes have occurred which render the cell less likely to respond to environmental changes by separation from its fellows. The phases characterized by an independent existence have been subordinated. Especially is this true of the flagellate phase, which in some lines, such as the Zygnemaceae, has dropped entirely out of the ordinary life cycle. That a particular phase does not regularly appear in the life cycle is not, to be sure, proof of the total loss of the potentialities necessary to its appearance. This fact is shown by the ability of the cells of Tetraspora and Stigeoclonium under some conditions to pass into an amoeboid phase in which they are capable of holozoic nutrition.

The type of coenobic colonial plant that proved best adapted to further evolution on this planet involves the continued close contact of sister cells after each of a series of divisions. The existence of the colony, in general, depends upon the formation of a persistent rigid layer between the sister cells at the time of, or shortly after, their formation. This layer may in some cases constitute the final thickness of the partition wall. Much more commonly, probably, it is supplemented by the deposition of additional layers on either side; the original layer then corresponding, in history, position, and function, to the middle lamellae of the larger green plants. The formation of a persistent partition wall depends upon the ability to secrete, under definite conditions, a certain substance or certain substances. Sometimes the original layer is easily ruptured, as in the common yeasts. But in the evolution of persistently colonial plants the middle lamella, if once fragile, has become more stable.

In a coenobe, such as Spirogyra, every vegetative cell has, throughout its active life, the full range of potentialities of every other cell. Any cell may remain vegetative, grow, and divide, or may become a gamete or an azygospore. When, in a coenobe, a cell responds to particular stimuli by taking on the characters of a gamete or of a spore, it becomes differentiated from the vegetative cells; but it can still transmit its full original equipment of potentialities to its offspring although it may itself be debarred

from expressing certain potentialities. A similar limitation, it has been seen, may be experienced by a one-celled organism when it has passed into particular phases. It follows that there is no difference in inherent potentialities as between the constituent cells of a coenobe. Harper has shown that in Hydrodictyon the potentialities of the equipotent cells determine, with the interaction of the environment, the character of a comparatively large and definitely constituted plant. He has shown the same to be true of Pediastrum in which some differentiation of cells appears, in well as of so complex a plant as Dictyostelium with its extensive cellular differentiation.

IV

The transition from coenobes to plants with differentiated cells, like that from unicellular organisms to coenobes, is gradual. Differentiation in structure and function implies the expression by different cells of different groups of potentialities; it results directly, as is obvious in the filamentous algae and fungi, from differences in the conditions surrounding the respective cells. All the cells of the plant are still equipotent; and any cell possesses all the potentialities of all the cells—it is totipotent—as is shown by the fact that any cell, at least while young, may give rise, in one way or another, to a complete new plant. The potentialities common to all the cells are, as it were, under the influence of the environment, sorted out and arranged into alternative life cycles; or it would be better now to say *life histories*, since some of the alternatives may not lead to reproduction and hence to a repetition of the story. But the differentiation takes place in the life of each cell. All the cells are, at the start and throughout at least most of their history, alike in their potentialities; all are, in Weismann's phrase, potentially immortal.

There may come a time in the history of a vegetative cell when it can no longer divide, and hence can under no circumstances originate a new plant. Obviously the power of reproduction is lost in the changes which precede the death of the cell; but it is possible that this loss, in some types of cells, anticipates the appearance of any degenerative changes. Certain of the alternative histories may, therefore, lead in time to a condition in which the cells upon which those alternatives were forced lose their immortality. One-celled organisms may likewise, in some environments, pass into a condition in which reproduction can not occur. The possibility of a condition of this sort is thus not new to the constituent cells of a many-celled organism; but in the latter case some of the cells necessarily lose their reproductive power as a result of their position in the plant.

Some degree of differentiation must result from a mere increase in size of the colony; for the larger the colony the more varied are the conditions to which its different cells are exposed. If, however, cellular differentiation were a function of size alone, Rhizoclonium and Draparnaldia would

be about equally differentiated. Differentiation beyond its most elementary stages evidently depends also, and more largely, upon an increasing susceptibility of cells to relatively slight differences in stimuli; the increased susceptibility brings about the expression of noticeably different potentialities by cells differently situated. Probably also quite early in evolutionary development—very certainly on a large scale at a later stage—there is an increase in the range of potentialities at the disposal of the cell, and hence also in the variety of possible life histories.

The secretion of a persistent partition wall between sister cells makes a colony possible, but of course does not determine the form of the colony. The form, whether unbranched or branched, filamentous or plate-like, depends in part upon the expression of various potentialities concerned with the relative growth of each cell in its respective axes and with the plane or planes in which division occurs. Some of these potentialities are among the new evolutionary developments; and, since species differ in these potentialities, they differ in their characteristic forms.

V

When, in evolutionary course, the potentialities appeared whose expression resulted in the development of a massive plant—one with cell divisions in more than two planes—the possibility of differentiated cellular development led to the formation of tissues. The internal cells of a massive plant are shielded from the immediate influence of conditions outside the plant, although indirectly, of course, still much affected by those conditions. The internal cells are subjected to an environment, a very important and immediately influential part of which consists of the surrounding cells. The variety of stimuli, including pressures and tensions and electrical and chemical changes, which act upon different cells of the plant is thus greatly increased, and there is made possible an increase in the variety of cellular differentiations. It is to be expected, then, that, quite apart from the acquisition of new potentialities, the arrangement of potentialities into alternative life histories should be carried further than in a filamentous or plate-shaped plant whose cells, while by no means uninfluenced by stimuli proceeding from neighboring cells, are all alike in touch with the world outside. Any increase in the size and complexity of the plant magnifies the importance of the interaction of its cells. In a large measure, as Goodrich has expressed it, the higher organisms have gradually substituted internal for external stimuli.

Every newly formed cell of a massive plant is embryonic, in the sense that it is capable of division. In general, an embryonic cell has certain structural characters; but the presence or absence of these characters does not affect its essentially embryonic nature. By the division of the embryonic cells in a primary meristem, followed by later divisions of some of their still embryonic derivatives, all the cells are provided which are to

constitute the tissues of the plant. The individual destiny of the cells formed in the meristem is evidently in no sense predetermined. Whether any such cell is to become a highly differentiated element of the xylem, phloem, or epidermis, or is to retain its embryonic character as an element of the meristem, cambium, pericycle, or cortex, depends upon its position, and therefore upon the stimuli which affect it. The same statement holds for the cells formed in the cambium or in any other embryonic region. A material alteration of conditions, as by a wound, by the incursion of a parasitic fungus, or even by a change in the position of the plant, may result in radically changing the course of development of a cell, even after it is well started upon the processes of differentiation. The life history of the cell, under the changed conditions, is shifted to a course quite different from that which a cell in its position would have followed under ordinary circumstances. The facts of development under both ordinary and exceptional conditions thus indicate that all the cells of a complex as of a simpler plant are, at least while embryonic, equipotent and totipotent.

A special case of a shift in the life history of a cell or of a group of cells as a result of changed conditions is seen when new organs or new plants are produced by regeneration. So extensive is the power of regeneration in bryophytes that one is almost tempted to say that any cell, at any time during its active existence, can give rise to a complete plant. In the vascular plants the power of regeneration is widespread also; witness the varied forms of vegetative multiplication, regeneration from leaves, the production of adventitious shoots and roots. In some cases regeneration involves the dedifferentiation of cells already well advanced in their developmental history. But it seems clear—though perhaps not yet rigidly demonstrated—that in a vascular plant each type of differentiating cell may reach a point in the course of its development beyond which, although it is still alive and functional, dedifferentiation and division are impossible. It is evident that such a state has been attained by "mature"—that is, dead—tracheids and vessels, as well as by the still living but enucleate cells of sieve tubes; but it seems to be true that in these and in other tissue cells there are conditions—among others, much thickened walls—which effectively inhibit division even before differentiation has run its full course.

The statement of the primary totipotence of all the cells of a plant must be modified by the admission that occasional irregularities in the working of the mitotic mechanism may result in an unequal distribution of potentialities. There is both cytological and genetic evidence that these mitotic accidents occur, perhaps not infrequently. But the possibility of such disturbances does not affect the validity of the general law of equi- and totipotence.

Among the metazoa an unequal distribution of potentialities is perhaps regularly affected in some cases; for example, by a discarding of part of

the chromosomal substance, as in certain species of Ascaris, or, as in some insects, by the inclusion in certain cells of particular cytoplasmic substances. If, and so far as, these occurrences effect a differential distribution of cellular potentialities, rather than something like a stimulus to a particular cellular development, they constitute deviations from the condition of primary totipotence which is probably as truly the general rule for the cells of animals as it is for the cells of plants.

The potentialities whose manifestation marks each particular type of cell in a vascular plant come to expression—under ordinary conditions—in a definite order. This orderly development seems to be determined in part by an inherited tendency to pass through a definite sequence of phases—a tendency which now applies to all the alternative life histories upon one of which the cell has embarked. But the orderly development is also partly dependent upon a progressively changing environment, which consists largely of other cells among which the one in question is firmly held. The importance of the environment is shown by the fact that, although the course of development of any cell of a massive plant seems to be less easily diverted to an alternative course than is that of a protist or of a cell of a coenobe, the cellular life history is, nevertheless, not rigidly fixed. In some cases, and for a limited time at least, it is subject to reversal—dedifferentiation. It is capable for a considerable time of being greatly modified by the onset of changed conditions. The stimulus that causes the formation of a gall may determine the expression of potentialities that otherwise would not be manifested either in the organ affected or in any other part of the plant. Thus the whole range of cellular potentialities may not be exhibited in a plant living under what we call "normal" conditions. To comprehend the extent of this range it is necessary to observe the plant under all conditions, including pathogenic, that it is capable of enduring.

In the course of the development of an embryonic cell into a differentiated tissue element, the structural peculiarities that usually characterize embryonic cells are modified. But the cell may still remain essentially embryonic. Next, perhaps, it becomes itself incapable of taking on characters other than those proper to the cells of the tissue of which it has become a part; but, so long as it retains the power of dividing, it can still transmit to its offspring its full initial complement of potentialities. Finally it may, and in many cases probably does, while still alive and otherwise functional, lose all power of division, and therewith its embryonic character.

The story that is presented, therefore, in ontogenetic development is that of a plant, simple or complex, composed of totipotent cells all beginning life with the power of transmitting their totipotence to their offspring. However complex the plant, many cells retain this power, remaining embryonic until they divide or become moribund. Others, undergoing marked changes in structure and in function, may in time lose their totipotence and their power of division. But so long as they can divide, they can give

rise to totipotent offspring. In this history there appears neither a fundamental distinction between "germ" and "somatic" cells, nor a progressive distribution of potentialities during ontogeny to differentiating groups of tissue elements. These two conceptions, it may be said in digression, have found some seeming justification in animal embryology; partly by the unequal distribution of particular nuclear or cytoplasmic substances already referred to; and especially by the fact that tissue development in the more complex metazoa involves a progressive differentiation extending through a series of cell generations. The history in these organisms, under ordinary conditions, from obviously embryonic cell to fully differentiated tissue element includes the terms of existence of a succession of cells, being in this respect somewhat comparable with the less strictly defined life cycle of a many-phased flagellate; whereas in a plant the corresponding history is mainly or wholly included within the lifetime of a single cell. But despite this important difference, it is probable that the fundamental ontogenetic processes of the metazoa do not differ in principle from those here outlined for the vascular plants. The culture of isolated animal tissues promises to supply important evidence upon this question.

The evolution of complex plants seems to have involved the loss of some of the potentialities that characterized their flagellate ancestors. The ability to revert to a flagellate phase, as shown in the formation of swarm-spores or motile gametes, has itself been lost—so far as we know—by the cells of conifers and angiosperms. It is noteworthy that in many ascending series, including those which led to the seed plants, this power was so long retained, even after incalculable ages of life upon land. The ability to pass into a naked amoeboid phase, still present in some of the Chlorophyceae, has also—again so far as we know—been lost by the cells of the more complex green plants. But if a few potentialities may be shown to have been lost, more have been gained. The widely varied types of cells found in the usually developed tissues of an oak, not to mention those of pathological growths, represent a vast number of potentialities which every newly formed cell of the tree possesses. Few of these potentialities, apart from those concerned in the more fundamental activities, were possessed by the oak's flagellate ancestors. The overwhelming majority have been added to the heritage of the cell. The acquisition of numerous new potentialities has characterized the evolution of the cells of complex organisms, both plant and animal.

The responses which consist in the seriated expression of potentialities characteristic of the cells of any tissue are adjusted with remarkable delicacy to what must be minute differences between the stimuli that incite them. This delicacy of adjustment to varying stimuli is apparent if one considers two adjoining cells, starting with the same inheritance; one becoming, perhaps, an element of the cortical parenchyma, another a bast fiber or a sieve-tube element. It follows that, besides many new po-

tentialities, the cell has acquired in the course of its evolution a susceptibility to differences in stimuli probably far beyond that possessed by any algal cell. This is another important feature in the evolution of complex organisms.

The net result of the delicately adjusted responses to almost innumerable stimuli—including particularly those due to the presence and activities of other cells in the same plant—is not only the orderly development of each cell but also a coördination in the development of all the cells. The coördinated responses of individual cells bring about "regulated" growth and development. The totality of cellular development, thus coördinated, is called the life history of the organism. It is not surprising that some investigators, viewing the history of the organism as a whole rather than as made up of the histories of individual cells, and bewildered by the complexity of development thus considered, have expressed their despair by the coining of magic words or phrases, intended to express the inexpressible, to explain the inexplicable. This is a needless confession of hopelessness. It is reasonable, and immeasurably more profitable, to assume that regulation can be explained, by a painstaking analysis of the stimuli at work in ontogenetic development, of the potentialities whose expression is conditioned by these stimuli, and of the cellular organization out of which the potentialities arise.

By means of such an analysis in terms of the individual cell, organic evolution must likewise ultimately be explained. Theories of the causes and of the course of evolution have been developed, in the main, in terms of the organism. All their sound elements can easily be restated in terms of the cell. Current discussions of evolutionary problems demonstrate that no material further progress is to be made by the use of the old terms and phrases. Consider, for example, the time and energy that are being wasted in controversies over the nature, the inheritance, and the modification of "congenital" and "acquired" characters, in which the characters are treated as qualities of the organism as a whole or of its constituent organs. There are no "characters" of an organ, still less of an organism, save in a figurative or abstract sense. What concretely exists and is inherited or modified from generation to generation is the fundamental constitution of the living matter, giving rise to certain potentialities and in some degree limiting and directing their expression. The organism is the resultant of the expression by each cell, under the conditions set by its constitution and by its environment, of some of its inherent potentialities.